ANALYSIS OF SECONDARY METABOLITE COMPOUNDS OF ETHYL ACETATE FRACTION AND CHLOROFORM FRACTION OF PARIJOTO FRUIT (Medinilla speciosa B) USING GC-MS

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Abstract. Background: Parijoto fruit (Medinilla speciosa Blume) is an endemic fruit around Mount Muria, Kudus, Central Java. Local people utilise parijoto as a traditional herbal medicine. The utilisation is due to the content of metabolite compounds, such as tannins, flavonoids, phenolics, terpenoids, and others. In order for parijoto fruit to be utilised properly, appropriate extraction and fractionation methods are needed. Thus, the bioactive compounds of parijoto fruit can be extracted optimally. The purpose of this study was to analyse the content of secondary metabolite compounds in methanol extract of parijoto fruit (Medinilla speciosa Blume) ethyl acetate fraction and chloroform fraction. Methods: The method used is processing simplisia until dry simplisia is obtained and extracted using maceration method and liquid-liquid fractionation using ethyl acetate fraction and chloroform fraction to obtain these fractions. Test analysis using Gas Chromatography-Mass Spectrometry (GC- MS). Results: Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanol extract of ethyl acetate fraction has more active compounds than methanol extract of chloroform fraction. The active compounds of methanol extract of ethyl acetate fraction are dimethyl sulfate, pentanoic acid, hexadecanoic acid, octadecanoic acid, 8-cyclohexadecen-1-one, 10-undecenal, tetracosanoic acid, eicosanoic acid, 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) phthalate, and cyclopentanethiol. The active compounds of chloroform fraction methanol extract are glycine, pyridine, aldosterone, combretastatin, and cobalt. Conclusion: There are secondary metabolite compounds in methanol extract of ethyl acetate fraction and chloroform fraction, namely 1,2-benzenedicarboxylic acid and bis(2-ethylhexyl) phthalate (30.59% area), pyridine, aldosterone, combretastatin, and cobalt (21.55% area), octadecanoic acid (12,49% area), cyclopentanethiol (11.40% area), glycine (9.84% area), hexadecanoic acid (9.14% area), 10-undecenal (8.75% area), tetracosanoic acid and eicosanoic acid (4.21% area), dimethyl sulfate and pentanoic acid (3.74% area), and 8-cyclohexadecen-1-one (1.75% area).

Key words: [Parijoto fruit (Medinilla speciosa Blume), Methanol, Ethyl acetate, Chloroform, Gas Chromatography-Mass Spectrometry (GC-MS)]

INTRODUCTION

The parijoto plant (Medinilla speciosa Blume) is a plant of the genus Medinilla that grows in the tropics. This plant is a typical plant from Colo Village, Kudus Regency, Central Java. Colo village is located on the slopes of Muria hill, which is one of the hills on Mount Muria which has an altitude of more than 1600 metres (Damayanti et al., 2023). Parijoto (Medinilla speciosa Blume) is one of the plants that is widely used by the people of the Kudus area as a medicinal plant. People generally consume Parijoto to treat thrush, diarrhoea, anti-inflammatory, antibacterial, and lower cholesterol (Damayanti et al., 2023).

Parijoto plants contain flavonoids, cardenolone, saponins (especially in fruits) and tannins (especially in leaves) (Niswah, 2014). The results showed that parijoto is a plant known to contain tannins, flavonoids, saponins, and glycosides in its fruit and has antioxidant and antibacterial activities. Tannins, flavonoids, and saponins are known as compounds that can be used as antioxidants and antibacterials (Niswah, 2014).

The parijoto plant is a typical shrub, its leaves are curved, single, and crossed opposite. The fruit is soft light purple, if it ages it becomes blackish purple and tastes sour and astringent. Parijoto leaves can be an anti-inflammatory drug and the fruit can be used as a medicine for thrush (Niswah, 2014). Parijoto leaves taste sour, bitter, and are refreshing because parijoto fruit contains saponins, cardenolone, and flavonoids, while the leaves contain saponins, cardenolone, and tannins (Niswah, 2014).

Parijoto plants contain many compounds that are beneficial to humans. For this reason, it is necessary to further explore the bioactivity of the chemical compound content of the Parijoto plant. This is the background of research on the content of compounds in parijoto fruit.

METHODS

Type of Research

This research is an experimental research of quantitative description which means data obtained from the examination of laboratory data with the results of numbers and images described in the discussion and taken from the conclusion.

Research Population

Population is a subject and object that has certain qualities and characteristics determined by the researcher and then conclusions can be drawn (Wahono, 2017). The population used is parijoto fruit (Medinilla speciosa Blume) obtained from Colo Village, Kudus Regency.

Research Sample

The sample is part of the number and characteristics possessed by the specified population (Jasmalinda, 2021). The research sample used is parijoto fruit (Medinilla speciosa Blume) which has been dried and mashed.

Location and Time of Research

The manufacture of extracts and fractions was carried out at the Microbiology Laboratory of the Cendekia Utama Kudus Institute of Health Technology, Research on the analysis of the compound content of the ethyl acetate and chloroform fractions by Gas Chromatography-Mass Spectrometry (GC-MS) method was carried out at the Integrated Laboratory of the Islamic University of Indonesia Yogyakarta. Research on the analysis of compound content of ethyl acetate and chloroform fractions by Gas Chromatography-Mass Spectrometry (GC-MS) method was conducted from March to July 2024.

Tools and Materials

The tools used in this research are digital scales, oven, blender, mesh sieve no 40, test tube, tube rack, filter paper, measuring cup (pyrex), separatory funnel, beaker glass, erlenmeyer, stirring rod, volume pipette, aluminium foil, moisture balance, rotary evaporator, a set of GC-MS. The main material used in this research is parijoto fruit obtained from Colo Village, Dawe District, Kudus Regency. Chemicals used for fractionation are ethyl acetate p.a, chloroform p.a, methanol, distilled water. Chemicals used for phytochemical screening were HCl 1%, HCl 0.1 N, concentrated HCl, NaOH 1 N, Mg powder, $FeCl_3$, and $FeCl_3$ 1%.

Plant Determination

Determination is done to determine the morphological characteristics of a plant and avoid errors in plant collection. Sample determination was carried out at the Ahmad Dahlan University Laboratory, Yogyakarta.

Parijoto Fruit Collection

Before extracting parijoto fruit (Medinilla speciosa Blume) as much as 2 Kg, wet sorting is carried out by separating dirt or foreign objects attached with clean running water.

Parijoto Fruit Extraction

Parijoto fruit as much as 1.5 kg was dried in a drying cabinet with a vulnerable time of 1 to 3 days. The results of drying are then calculated the weight of the drying shrinkage rate. Parijoto fruit was mashed using a blender and sieved using mesh sieve number 40, then the maceration process was carried out using methanol solvent as much as 1: 7 stored in a clear glass jar closed using aluminium foil. The resulting macerate was filtered using flannel cloth and rinsed with methanol. Then thickened with a rotary evaporator at 120 rpm and a temperature of 40 ° C and concentrated on a waterbath until concentrated. Then weighed the weight of the extract

Ethyl Acetate and Chloroform Fractionation

Fractionation of methanol extract of parijoto fruit (Medinilla speciosa Blume) by split funnel method. Methanol extract was added with distilled water and ethyl acetate solvent 1:1:1 (v/v/v) into a separatory funnel, homogenised and allowed to separate for 10-15 minutes until there were 2 layers between methanol and ethyl acetate. The methanol layer at the bottom and the ethyl acetate p.a layer at the top. The separatory funnel tap was opened to separate the two layers. The fraction ethyl acetate p.a fraction was separated, then the bottom layer was put back into the separating funnel and added chloroform p.a solvent and the same method was carried out with the ethyl acetate fraction.

Replicated 3 times each ethyl acetate fraction p.a and chloroform fraction p.a. Performed until the extract solution becomes colourless (clear). All fractions obtained, evaporated with a rotary evaporator with a temperature of 40 ° C, then evaporated the remaining solution in the sample using a waterbath so as to obtain a thick fraction of ethyl acetate p.a and a thick fraction of chloroform p.a. Then tested using Gas Chromatography-Mass Spectrometry (GC-MS). (Rusli et al., 2023).

Phytochemical Screening Test

1) Alkaloid Test
   Each methanol extract of ethyl acetate fraction and chloroform fraction of parijoto fruit (Medinilla speciosa Blume) as much as 2 mL is put into a test tube added 2-3 drops of 1% HCL, The addition of positive results produces an orange red to brownish precipitate (Mahmiah et al., 2017).
2) Polyphenol Test
   Each methanol extract of the ethyl acetate fraction and chloroform fraction as much as 2 mL is put into a test tube and then heated on a water bath to boil, filtering in a hot state then after the cold FeCl3 is added the results show a black blue colour indicating a positive extract of polyphenols (Suratno, 2016).
3) Quinone Test
   Each methanol extract of ethyl acetate fraction and chloroform fraction was put in a test tube as much as 2 mL then added NaOH 1 N shaken for 1 minute. The reaction results are indicated by a yellow colour change (Lanipi et al., 2022).
4) Saponin Test
   Each methanol extract of ethyl acetate fraction and chloroform fraction as much as 2 mL was put into a test tube then added HCL 0.1 N shaken for 1 minute. ±A positive test is indicated by the formation of permanent foam for 15 minutes. Then the extract positively contains saponins (Illing et al., 2017).
5) Flavonoid Test
   Each methanol extract of ethyl acetate fraction and chloroform fraction as much as 2 mL was put into a test tube. Added 3-5 drops of concentrated HCl and added a little Mg powder. A positive reaction occurs when there is a change in pink, magenta colour, indicating that the sample is positive for flavonoids (Mahmiah et al., 2017).
6) Tannin Test
   Each methanol extract of ethyl acetate fraction and chloroform fraction as much as 2 mL was put into a test tube added 2-3 drops of FeCl3 1%. Positive results containing tannin are indicated by the appearance of a brownish green colour (Mahmiah et al., 2017).

Gas Chromatography-Mass Spectrometry (GC-MS) assay

A total of 1 µL of methanol extract of ethyl acetate fraction and chloroform fraction was injected in Gas Chromatography-Mass Spectrometry (GC-MS) for analysis of different compounds. Chromatography instruments and conditions were performed on a Gas Chromatography-Mass Spectrometry (GC-MS) HP 6890 system. The column used was capillary model number Agilent 19091S-433 HP-5MS 5% Phenyl Methyl Siloxane with a length of 30 m, diameter of 250 µm and thickness of 0.25 µm. The oven temperature used was between 100 and 220°C. The rate of temperature increase was 15°C with a flow rate of 1.0 mL per minute. The carrier gas used was helium with a pressure of 10.5 psi and a total rate of 140 mL per minute and a split ratio of 1:50. Eluted components will be detected on a mass detector. Spectrometry of known compound components will be stored in the NIST library and determine the name of the compound, the molecular weight included in the class of compounds such as alkaloids and flavonoids which are useful compound components for Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

RESULTS AND DISCUSSION

Plant Determination

Plants that will be studied before being used as simplisia are first determined. Determination is carried out with the aim of knowing the truth of the plants to be studied and avoiding errors in collecting materials and avoiding the possibility of mixing the plants to be studied with other plants (Maria et al., 2021). Determination of parijoto fruit (Medinilla speciosa Blume) was carried out at the Ahmad Dahlan University Laboratory, Yogyakarta. Based on the determination results, it was found that the fruit used in this study was true, namely parijoto fruit (Medinilla speciosa Blume).

Processing of Parijoto Fruit Simplisia (Medinilla speciosa Blume)

Parijoto fruit (Medinilla speciosa Blume) that is old or purplish red in colour is taken directly

from Colo Village, Dewe District, Kudus Regency, Central Java. Fresh parijoto fruit as much as 2 kg is done wet sorting to be separated from dirt or foreign objects, then washed with running water until clean, drained and dried until no water drips. Weighing the parijoto fruit (Medinilla speciosa Blume) produced 1.5 kg, then chopped in half (Niswah, 2014). The chopping of parijoto fruit (Medinilla speciosa Blume) is done to facilitate the drying process which aims to obtain non-perishable simplisia, so that it can be stored for a longer time by reducing water content and stopping enzymatic reactions that can reduce the quality or destroy simplisia (Parfati et al., 2018).

Drying of simplisia is carried out using an oven cabinet with a vulnerable time of 35 hours at a temperature of 40 ° C to produce 250 grams. Parijoto fruit (Medinilla speciosa Blume) after drying is then calculated the drying shrinkage and mashed using a blender until the fine simplisia becomes powder then sieved using mesh sieve number 40 which aims to separate coarse ingredients from fine ingredients in the process of filtering simplisia. The fine powder of parijoto fruit was then weighed to produce 200 grams of fine powder (Depkes RI, 1986).

Determination of Water Content

Simplisia that has been dried and mashed is then tested for water content in order to determine the residual water still contained in parijoto fruit powder (Medinilla speciosa Blume). The tool used to test water content is moisture balance (Depkes RI, 2000). The results of the water content obtained in parijoto fruit simplisia (Medinilla speciosa Blume) are in accordance with the quality requirements of 8% (Depkes RI, 1986). Too high water content will cause microbial growth which will reduce the stability of the extract. Too low moisture content can change the chemical composition (Utami, 2020).

Preparation of Viscous Extract

Simplisia parijoto fruit powder (Medinilla speciosa Blume) weighed as much as 100 grams was extracted by maceration method which has the advantage of simple procedures and equipment used, relatively low cost. The disadvantage of the maceration method is that it takes a relatively long time (Willian & Pardi, 2022). The extraction process of maceration method with 100 grams of powder is put in a clear jar, soaked with methanol as much as 700 mL using aluminium foil, every 12 hours stirred, placed in an airtight place for 5 days. The resulting macerate was filtered using flannel cloth and rinsed with methanol up to 1000 mL then thickened with a rotary evaporator with 120 rpm and a temperature of 40°C and concentrated on a waterbath until concentrated. The extract of parijoto fruit (Medinilla speciosa Blume) that has been obtained is then weighed and the yield value is determined.

Fractionation

Fractionation is the separation of compounds based on their polarity. A total of 40 grams of thick methanol extract of parijoto fruit was fractionated with ethyl acetate solvent using a split funnel, replicated 3 times until clear. Then the methanol extract of parijoto fruit was fractionated with chloroform, replicated 3 times until clear. The results obtained are ethyl acetate fraction and chloroform fraction. Fractionation is done to separate compounds based on their level of polarity. The ethyl acetate fraction is semi-polar and the chloroform fraction is non- polar (Ambarsari, 2014).

The principle of separation in the fractionation process is based on the difference in the level of polarity and the difference in specific gravity between the two fractions, namely the fraction that has a larger specific gravity will be in the lower phase, while the fraction that has a smaller specific gravity will be in the upper phase (Budilaksono et al., 2014).

The thick methanol extract of parijoto fruit (Medinilla speciosa Blume) was weighed as much as 40 grams dissolved with 40 mL of distilled water then fractionated with 40 mL of ethyl acetate p.a in a separatory funnel shaken occasionally the lid was opened then left until two layers were formed, the ethyl acetate fraction was taken and the ethyl acetate fraction p. a was put into an erlenmeyer and the methanol extract of parijoto fruit (Medinilla speciosa Blume) was re-fractionated using ethyl acetate p.a replicated 3 times until the ethyl acetate fraction formed.The ethyl acetate fraction was taken and the ethyl acetate fraction was put into Erlenmeyer and the methanol extract of parijoto fruit (Medinilla speciosa Blume) was re-fractionated using ethyl acetate p.a replicated 3 times until the ethyl acetate fraction was clear. The same thing was done in chloroform fractionation, 40 grams of thick methanol extract of parijoto fruit (Medinilla speciosa Blume) was dissolved with 40 mL of distilled water and then fractionated with chloroform p. a as much as 40 mL in a funnel.a as much as 40 mL in a separatory funnel shaken occasionally the lid is opened then left until two layers are formed, the chloroform fraction is taken and the chloroform fraction p.a is put into erlenmeyer and the methanol extract of parijoto fruit (Medinilla speciosa Blume) is re-fractionated using chloroform p.a replicated 3 times until chloroform

is clear.

Phytochemical Screening Test

Phytochemical screening of the ethyl acetate fraction and chloroform fraction of parijoto fruit (Medinilla speciosa Blume) was carried out to confirm the presence of alkaloid compounds, polyphenols, quinones, saponins, flavonoids, steroids, and triterpenoids.

Table 1. Phytochemical Screening Results of Ethyl Acetate Fraction of Parijoto Fruit (Medinilla speciosa Blume)

Group Compound	Reagent	Colour/Shape	Result
Alkaloids	HCl 1%	-	-
Polyphenols	$FeCl_2$	Blue-black	+
Quinones	NaOH 1N	Yellow	+
Saponin	HCl 0.1	Formation of foam	+
Glycosides	Liebermann Burchard + Sulfuric Acid p.	-	-
Glycosides	Molish+ Sulfuric Acid p.	Formation of purple ring at liquid boundary	+
Flavonoids	Concentrated HCl + Mg powder	Pink magenta	+
Tannin	$FeCl_3$ 1%	Brownish green	+

Table 2. Phytochemical Screening Results of Chloroform Fraction of Parijoto Fruit (Medinilla speciosa Blume)

Group Compound	Reagent	Colour/Shape	Result
Alkaloids	HCl 1%	-	-
Polyphenols	$FeCl_2$	Blue-black	+
Quinones	NaOH 1N	Yellow	+
Saponin	HCl 0.1	-	-
Glycosides	Liebermann Burchard + Sulfuric Acid p.	-	-
Glycosides	Molish+ Sulfuric Acid p.	-	-
Flavonoids	Concentrated HCl + Mg powder	-	-
Tannin	$FeCl_3$ 1%	-	-

1) Alkaloids

Based on the results of phytochemical screening, it is known that the ethyl acetate fraction and chloroform fraction of parijoto fruit (Medinilla speciosa Blume) using 1% HCL reagent on the sample formed a pink colour, the results show that the methanol fraction of parijoto fruit (Medinilla speciosa Blume) negatively does not contain alkaloid compounds (Mahmiah et al., 2017).

2) Polyphenols

Polyphenol phytochemical screening test by heating on a water bath to boiling, and filtering in a hot state after cooling, $FeCl_3$ reagent is added to form a black blue colour, the results show that the ethyl acetate fraction and chloroform fraction of parijoto fruit (Medinilla speciosa Blume) contain polyphenolic compounds (Habibi et al., 2018).

3) Quinone

Quinone phytochemical screening test on the ethyl acetate fraction and chloroform fraction added NaOH reagent 1 N shaken for 1 minute there is a yellow colour change, the results show positive quinone compounds contained in the ethyl acetate fraction and chloroform fraction of parijoto fruit (Medinilla speciosa Blume) (Lanipi et al., 2022). Quinone compounds have the ability as antibiotics and painkillers and stimulate new cell growth in the skin (Noer & Pratiwi, 2016).

4) Saponins

The saponin phytochemical test showed a positive reaction due to the formation of permanent foam on the addition of 0.1 N HCL reagent, the results showed the presence of saponin compounds in the ethyl acetate fraction of parijoto fruit (Medinilla speciosa Blume). Saponins are commonly known as non-volatile compounds and are very soluble in water (cold or hot) and alcohol, but form colloidal foam in water and have good detergent properties (Putri et al., 2023).

5) Glycosides

a. Liebermann Burchard reagent

The glycoside screening test uses the Liebermann Burchard reagent method. Lieberman Burchard reagent is a mixture of anhydrous cetic acid and concentrated sulfuric acid (Sahriawati et al., 2014). Glycoside screening test using anhydrous acetic acid reagent p and sulfuric acid p if there is a blue or green colour change then parijoto fruit (Medinilla speciosa Blume) ethyl acetate fraction contains Liebermann Burchard (Malik et al., 2016). The results of the study on the ethyl acetate fraction and chloroform fraction produced a clear colour negative then each fraction does not contain glycosides.

b. Molish reagent

Glycoside screening test using molish reagent method then added 2- 3 drops of sulfuric acid p. resulted in the occurrence of changes in the formation of a purple ring at the liquid boundary indicating the presence of glycoside compounds in parijoto fruit (Medinilla speciosa Blume) in the screening test on the ethyl acetate fraction showed the formation of a purple ring positively containing molish reagent glycosides. The results of the screening test on the chloroform fraction of the formation of a light pink ring precipitate negative does not contain molish glycoside compounds (Malik et al., 2016). Molish test is a qualitative chemical test for all types of carbohydrates. Molish test can be used on all types of carbohydrates. The appearance of a purple ring is a condensation between furtural or hydroxymethyl furfural with α-naphthol in molish reagent (Sarah, 2021).

6) Flavonoids

Flavonoids are tested by adding 3-5 drops of concentrated HCI and adding a little Mg so that flavonoid compounds will react with concentrated HCI and Mg so that the results show a magenta pink colour, indicating that the ethyl acetate fraction of parijoto fruit (Medinilla speciosa Blume) positively contains flavonoid compounds. The chloroform fraction shows a pink bottom layer of white sediment top layer negatively does not contain flavonoid compounds (Sulistyarini et al., 2019). The addition of concentrated HCL and Mg indicates the presence of flavonoid compounds due to the reduction of Mg and HCI resulting in a magenta pink colour (Sulistyarini et al., 2019).

7) Tannins

Tannin test by adding $FeCl_3$ 1%. Positive results containing tannins are indicated by the appearance of a brownish green colour (Mahmiah et al., 2017). Based on the results of phytochemical tests with $FeCl_3$ 1% methanol extract of parijoto fruit (Medinilla speciosa Blume) showed positive results containing tannin compounds, this is indicated by a blackish green colour. The formation of a blackish green colour in the extract after adding $FeCl_3$ 1% is because tannins will react with $FeCl_3$ 1%. Tannin compounds have many OH groups which cause them to be polar, so tannin compounds can dissolve in polar solvents such as methanol so that tannins can be extracted in methanol solvents (Halimu et al., 2020). The results of the screening test on the negative yellow chloroform fraction did not produce a colour containing tannin compounds in the chloroform fraction.

8) Triterpenoids and Steroids

Triterpenoid screening test with the addition of anhydrous acetic acid p. Positive results show a brownish red colour (Mahmiah et al., 2017). The results of the research reaction of methanol extracts and ethyl acetate and chloroform fractions did not show positive results because the foam obtained after shaking was not brownish red and greenish blue but the resulting colour was light pink or clear. Steroid screening test with the addition of H_2SO_4 positive results showed a brownish red colour. The results of the screening test in the study of ethyl acetate fractions and chloroform fractions are clear pink, meaning that the ethyl acetate fraction and chloroform fraction do not contain steroid compounds (Mahmiah et al., 2017). Identification and steroids are based on the ability of triterpenoid compounds to form colours by H_2SO_4 in anhydrous acetic acid solvents. The difference in colour produced by triterpenoids and steroids is due to differences in groups at the C-4 atom (Capinera, 2021).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis Test

The test results of parijoto fruit methanol extract (Medinilla speciosa Blume) ethyl acetate fraction and chloroform fraction using Gas Chromatography-Mass Spectrometry (GC-MS) showed that the compounds of parijoto fruit methanol extract (Medinilla speciosa Blume) ethyl acetate fraction were more than parijoto fruit methanol extract (Medinilla speciosa Blume) chloroform fraction.

1. Ethyl Acetate Fraction

In the methanol extract of parijoto fruit (Medinilla speciosa Blume) ethyl acetate fraction shows there are 12 peaks and 6 compounds that have uses or properties. The graph of Gas Chromatography-Mass Spectrometry (GC-MS) results of ethyl acetate fraction compounds can be seen in Figure 1.

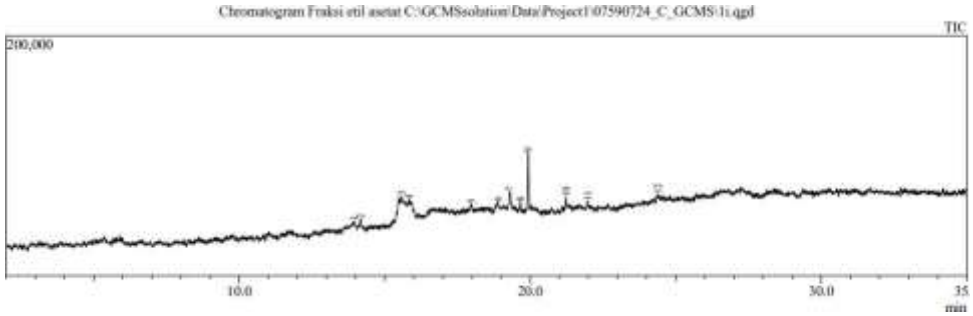
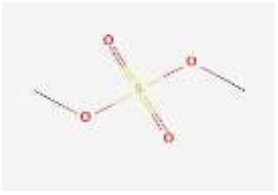
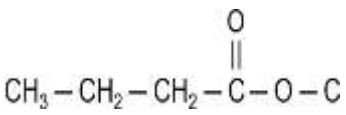


Figure 1. Graph of *Gas Chromatography-Mass Spectrometry* (GC-MS) Test Results of Methanol Extract of Ethyl Acetate Fraction

Compounds that have efficacy in methanol extract of parijoto fruit (Medinilla speciosa Blume) ethyl acetate fraction are located on peak 1, peak 2, peak 4, peak 6, peak 7, peak 8, peak 9, and peak 12 by producing 11 compounds namely dimethyl sulfate, pentanoic acid, hexadecanoic acid, octadecanoic acid, 8-cyclohexadecen-1-one, 10-undecenal, tetracosanoic acid, eicosanoic acid, 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) phthalate, and cyclopentanethiol.

The results of active compounds in the methanol extract of parijoto fruit (Medinilla speciosa Blume) ethyl acetate fraction can be seen in the following table.

Table 3. Gas Chromatography-Mass Spectrometry Peak 1 Test Results (GC-MS) Methanol Extract of Ethyl Acetate Fraction

Peak	Compound Name	Structure Chemical	Molecular Weight	% Area
1	dimethyl ester /dimethyl sulfate		$C_2H_6SO_4$	214	3,74
1	pentanoic acid /pentanoic acid / valeric acid		$C_5H_{10}O_2$	194	3,74

a) Dimethyl sulphate

Dimethyl sulphate is the dimethyl ester of sulfuric acid. It acts as an alkylating agent

and an immunosuppressive agent. Alkylating agents were the first nonhormonal drugs to be used effectively in cancer treatment. Immunosuppressive agents are drugs that suppress the immune system and reduce the risk of foreign body rejection such as transplanted organs (Penketh et al., 1990).

Dimethyl sulphate is used as a methylating agent in the manufacture of many organic chemicals. It is also used in the manufacture of dyes and perfumes, for the separation of mineral oils, and for the analysis of automotive fluids. Previously, dimethyl sulphate was used as a war gas (National Library of Medicine, 2024).

Used as a methylating agent in the synthesis of many organic compounds. Methylating agent for amines and phenols, polyurethane-based adhesives. Methylating agent and sulfate for agricultural chemicals, fabric softeners, dyes, synthetic drugs, and various intermediates (National Library of Medicine, 2024).

b) Pentanoic Acid

Pentanoic acid occurs naturally in some foods but is also used as a food additive. This compound has a pleasant odour and fruity taste and can therefore be applied in perfumes, cosmetics, and foodstuffs (Patocka et al., 2010). Pentanoic acid is also used as a sugarcane ripening agent and intermediate. Used in the manufacture of solvents, rodenticides, and fungicides. Present in various plants, fruits, dairy products, and meat (National Library of Medicine, 2024). Pentanoic acid in agriculture is commonly used in pesticides. Pesticides are chemical substance compounds used to control various pests on plants (National Library of Medicine, 2024). This compound is also an intermediary for flavours and perfumes, ester type lubricants, plasticizers, pharmaceuticals, vinyl stabilizers (National Library of Medicine, 2024).

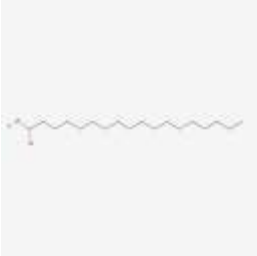
Table 4. Gas Chromatography-Mass Spectrometry Peak 2 Test Results (GC-MS) Methanol Extract Ethyl Acetate Fraction

Peak	Compound Name	Structure Chemical		Molecular Weight	% Area
2	Hexadecanoic Acid		$C_{16}H_{32}O_2$	270	9,14

In table 4 above is the result of peak 2 Gas Chromatography-Mass Spectrometry (GC-MS) test of methanol extract of ethyl fraction which produces 1 active compound, namely hexadecanoic acid. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The following is an explanation and properties or benefits of hexadecanoic acid.

Hexadonic Acid includes organic acids. Hexadecanoic acid is found as a glycerol ester in oils and fats; produced from palm oil, Japanese wax, or Chinese vegetable fats. A very common natural fatty acid; used to make palmitic and metallic esters; in soaps and cosmetics; in lubricating oils; for waterproofing coatings; in food-grade additives; as a non-drying oil or surface coating (National Library of Medicine, 2024). The compound is also used in the manufacture of metallic palmitate, soaps, lubricating oils, waterproofing materials, and food additives (Larranaga et al., 2016). There are industrial processes that risk exposure such as painting (pigments, binders, and biocides) (National Library of Medicine, 2024). In cosmetic preparations, especially soap preparations, hexadecanoic acid becomes a soap agent as a non-drying oil or surface coating (Ashford, 1994).

Table 5. Gas Chromatography-Mass Spectrometry Peak 4 Test Results (GC-MS) Methanol Extract Ethyl Acetate Fraction

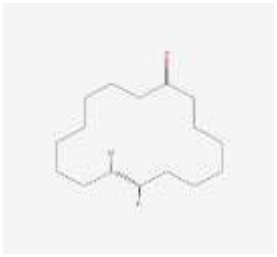
Peak	Compound Name	Structure Chemical	Molecular Weight	% Area
4	Octadecanoid Acid		$C_{18}H_{36}O_2$	298	12,49

In table 5 above is the result of peak 4 Gas Chromatography-Mass Spectrometry (GC-MS) test of methanol extract of ethyl fraction which produces 1 active compound, namely octadecanoic acid. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The following is an explanation and properties or benefits of octadecanoic acid.

Octadecanoic acid is used in cosmetics, pharmaceuticals, food additives, waterproofing agents, plastic stabilisers, emulsifiers, and rubber lubricants and dusting agents (National Library of Medicine, 2024). Octadecanoic acid is used as an intermediate for metallic soaps and oils, household soap products, synthetic rubber vulcanisation activator, and alkyd and epoxy resins for surface coatings; Used in cosmetic and pharmaceutical creams and lotions, waxes, phonograph records, dentistry materials, insulators, lubricants, shoe and metal polish, coatings, food packaging, and modelling compounds; used as a polymerisation emulsifier of synthetic rubbers, a release agent in baked goods and confectionery, a dispersing and softening agent in rubber compounds, paper adhesives (specialty papers), textile auxiliaries, to make stearates of metals such as aluminium and zinc (O'Neil et al., 2013).

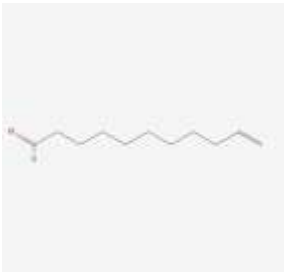
For suppositories, coating enteric pills, ointments and for coating bitter medicines. Manufacture of stearates from aluminium, zinc and other metals. Soap stearin for opodeldoc, wax, vinyl records, insulators, modelling compounds. Stain removal creams and other cosmetics. Stearic acid is an endogenously produced metabolite found in the human body. stearic acid is used in metabolic reactions, catabolic reactions, or waste production (O'Neil et al., 2013).

Table 6. Gas Chromatography-Mass Spectrometry Peak 6 Test Results (GC-MS) Methanol Extract Ethyl Acetate Fraction

Peak	Compound Name	Structure Chemical	Molecular Weight	% Area
6	8-Cyclohexadecen -1-one		$C_{16}H_{28}O$	236	1,75

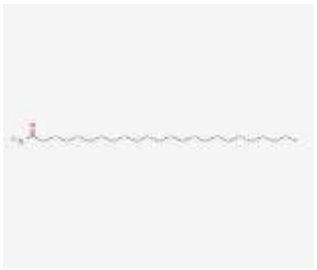
In table 6 above is the result of peak 6 Gas Chromatography-Mass Spectrometry (GC-MS) test of methanol extract of ethyl fraction which produces 1 active compound, namely 8-cyclohexadecen-1-one. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The compound 8- cyclohexadecen-1-one has several properties or benefits for human needs. 8-Cyclohexadecen-1-one is used as a fragrance and odour agent. The fragrances used can be for perfumes, cosmetics, and clothing (Enviromental Protection Agency, 2024).

Table 7. Gas Chromatography-Mass Spectrometry Peak 7 Test Results (GC-MS) Methanol Extract Ethyl Acetate Fraction

Peak	Compound Name	Structure Chemical	Molecular Weight	% Area
7	10-Undecenal		$C_{11}H_{20}O$	168	8,75

In table 7 above is the result of peak 7 Gas Chromatography-Mass Spectrometry (GC-MS) test of methanol extract of ethyl fraction which produces 1 active compound, namely 10-Undecenal. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The compound 10-Undecenal has properties or benefits for human life. 10-Undecenal has the benefit of being a household cleaning and care product, for example, air fresheners, home air fresheners, including candles with fragrances (Dionisio et al., 2018). Food additives (flavouring ingredients). Flavouring is a food additive that gives flavour to certain ingredients, so that a food can be more sweet, sour, and so on. Generally, flavouring is given to foods that do not or lack flavour such as jelly, soupy dishes, and so on (National Library of Medicine, 2024).

Table 8. Gas Chromatography-Mass Spectrometry Peak 8 Test Results (GC-MS) Methanol Extract Ethyl Acetate Fraction

Peak	Compound Name	Structure Chemical	Molecular Weight	% Area
8	Tetracosanoic Acid		$C_{24}H_{48}O_2$	382	4,21
8	Eicosanoic Acid		$C_{20}H_{40}O_2$	326	4,21

In table 8 above is the result of peak 8 Gas Chromatography-Mass Spectrometry (GC-MS) test of methanol extract of ethyl fraction which produces 2 active compounds, namely tetracosanoic acid and eicosanoic acid. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The following is an explanation and properties or benefits of tetracosanoic acid and eicosanoic acid.

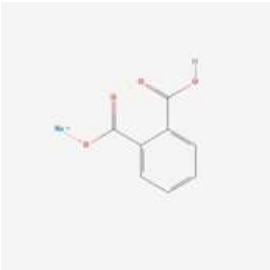
a. Tetracosanoic Acid

Tetracosanoic acid is a C24 straight-chain saturated fatty acid. It is a component of essential oils, plant metabolites, human metabolites, and Daphnia tenebrosa metabolites (Chemical Entities of Biological Interest, 2024). It is a very long-chain fatty acid and a straight-chain saturated fatty acid. It is the conjugate acid of tetracosanoic acid. Tetracosanoic acid is also used as a softener and conditioner (National Library of Medicine, 2024).

b. Eicosanoic Acid

Eicosanoic acid can be used as a cleanser, emulsifier, blurring agent, and surfactant. Eicosanoic acid can also be utilised in cosmetic preparations as an emulsifier (National Library of Medicine, 2024).

Table 8. Gas Chromatography-Mass Spectrometry Peak 9 Test Results (GC-MS) Methanol Extract Ethyl Acetate Fraction

Peak	Compound Name	Structure Chemical	Molecular Weight	% Area
9	1,2-Benzenedicarboxylic acid / Phthalic acid		$C_8H_5NAO_4$ 390	30,59
9	Bis(2- ethylhexyl) phthalate		$C_{24}H_{38}O_4$ 390	30,59

In table 9 above is the result of peak 9 Gas Chromatography-Mass Spectrometry (GC-MS) test of methanol extract of ethyl fraction which produces 2 active compounds namely 1,2-benzenedicarboxylic acid and bis(2-ethylhexyl) phthalate. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The following is an explanation and properties or benefits of 1,2- benzenedicarboxylic acid and bis(2-ethylhexyl) phthalate.

a. 1,2-Benzenedicarboxylic acid

Used in household cleaning and care products: laundry and fabric care: dry cleaner. Also used as a dye, phenolphthalein, phthalimide, anthranilic acid, synthetic perfume, and laboratory reagent (National Library of Medicine, 2024). Environmental transformation as a product of pesticide transformation. Pesticides are materials that are widely used in various sectors, especially agriculture/plantations, fisheries, and agriculture. The use of pesticides in the forestry, food, and agriculture sectors aims to eliminate nuisance plants, fungi, insects, rodents, and other organisms (National Library of Medicine, 2024).

b. Bis(2-ethylhexyl) phthalate

This compound can be used to cover typed or inked text on paper allowing new text to be superimposed. Products used for cleaning or safety in work or industrial environments e.g. industrial cleaning supplies or laundry detergents, spill containment supplies (National Library of Medicine, 2024). Materials used for construction e.g. flooring, tiles, sinks, bathtubs, mirrors, wall/drywall materials, wall-to-wall carpeting, insulation, playground surfaces, including semi-permanent fixtures such as taps and lights (National Library of Medicine, 2024). Can be used as an adhesive including all-purpose glue, superglue and epoxy; excludes wood glue. Used in putty/sealant: A liquid or gel designed to seal cracks or fill cracks and depressions in hard surfaces (National Library of Medicine, 2024).

Table 10. Gas Chromatography-Mass Spectrometry Peak 12 Test Results (GC-MS) Methanol Extract Ethyl Acetate Fraction

Peak	Compound Name	Structure Chemical	Molecular Weight	% Area
12	Cyclopentanethiol		$C_5H_{10}S$ 116	11,40

In table 10 above is the result of peak 12 Gas Chromatography-Mass Spectrometry (GC-MS) test of methanol extract of ethyl fraction which produces 2 active compounds, namely cyclopentanethiol. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The following is an explanation and properties or benefits of cyclopentanethiol.

Cyclopentanethiol is commonly used in food additives (flavouring ingredients) and food seasonings. Flavouring is a food additive that gives flavour to certain ingredients, so that a food can be sweet, sour, and so on (National Library of Medicine, 2024). Generally, flavouring is given to foods that do not or lack flavour (e.g. jelly, soupy dishes, etc.) so that it is preferred by consumers (National Library of Medicine, 2024).

In the Gas Chromatography-Mass Spectrometry (GC-MS) analysis test, the methanol extract of the ethyl acetate fraction produced 8 peaks that had active compounds, namely peak 1, peak 2, peak 4, peak 6, peak 7, peak 8, peak 9, and peak 12. Peak 1, peak 8, and peak 9 because they produce 2 active compounds each. Peak 1 produces active compounds namely dimethyl sulfate and pentanoic acid. Peak 8 produces active compounds, namely tetracosanoic acid and eicosanoic acid. Peak 9 produces active compounds namely 1,2-benzenedicarboxylic acid and bis(2-ethylhexyl) phthalate. The peak that has the highest percent area is peak 12 (11.40% area) by producing cyclopentanethiol compounds

2. Chloroform Fraction

In the methanol extract of parijoto fruit (Medinilla speciosa Blume) chloroform fraction showed 5 peaks and 5 compounds that have uses or properties. The graph of Gas Chromatography-Mass Spectrometry (GC-MS) results of ethyl acetate fraction compounds can be seen in Figure 2.

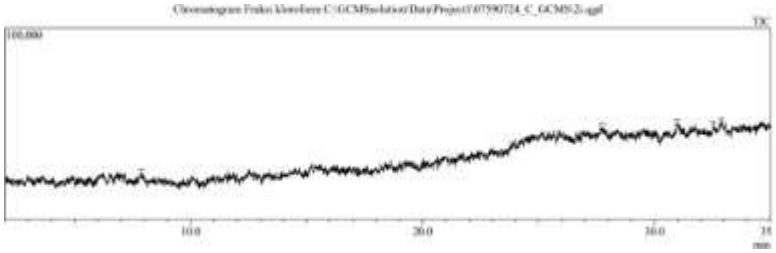
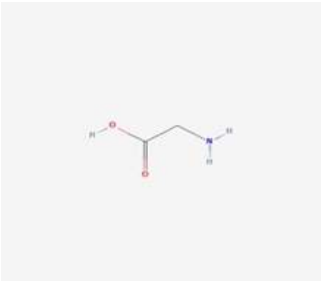


Figure 2. Graph of Gas Chromatography-Mass Spectrometry Test Results (GC-MS) Methanol Extract Chloroform Fraction

Compounds that have efficacy in methanol extract of parijoto fruit (Medinilla speciosa Blume) chloroform fraction are located in peak 2 which produces glycine compounds and in peak 3 which produces pyridine, aldosterone, combretastatin, and cobalt compounds.

The results of active compounds in the methanol extract of parijoto fruit (Medinilla speciosa Blume) chloroform fraction can be seen in the following table.

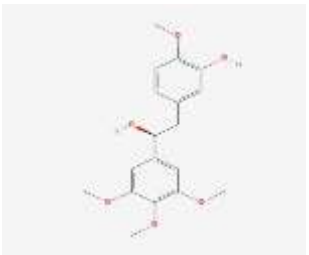
Table 11. Gas Chromatography-Mass Spectrometry Peak 2 Test Results (GC-MS) Methanol Extract of Chloroform Fraction

Peak	Compound Name	Structure Chemical		Molecular Weight	% Area
2	Glycine		$C_2H_5NO_2$	607	9,84

In table 11 above is the result of peak 2 Gas Chromatography-Mass Spectrometry (GC- MS) test of methanol extract of ethyl fraction which produces 1 active compound glycine. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The following is an explanation and properties or benefits of glycine.

Glycine is a non-essential amino acid. This amino acid is found mainly in gelatin and silk fibroin and is used therapeutically as a nutrient. This amino acid is also a rapid inhibitory neurotransmitter (NLM, 2024). Glycine is an essential component and precursor to many macromolecules in cells. Glycine is involved in the body's production of DNA, phospholipids, and collagen, as well as in the release of energy (Van Hove et al., 2005).

Table 8. Gas Chromatography-Mass Spectrometry Peak 3 Test Results (GC-MS) Methanol Extract Chloroform Fraction

Peak	Compound Name	Structure Chemical		Molecular Weight	% Area
2	Pyridine		C_5H_5N	296	9,84
2	Aldosterone		$C_{21}H_{28}O_5$	504	9,84
2	Combretastin		$C_{18}H_{22}O_6$	310	9.84

2	Cobalt		Co	348	9,84

In table 4.15 above is the result of peak 3 Gas Chromatography-Mass Spectrometry (GC-MS) test of methanol chloroform extract which produces 4 active compounds namely pyridine, aldosterone, combrestatin, cobalt. Included are the chemical structure, chemical formula, molecular weight, and percent area of the active compound. The following is an explanation and properties or benefits of pyridine, aldosterone, combrestatin, and cobalt.

a. Pyridine

Used as a solvent for paints and rubber; used as an intermediate for medicines, dyes, pesticides, textile coatings, and other chemicals; also used as a flavouring agent (National Library of Medicine, 2024). Vitamin products (especially nicotinic acid), sulfa drugs, disinfectants, colouring agents, explosives; used in the rubber industry (Browning, 1965). Diquat & paraquat, piperidine, water-retaining agents used in textiles; solvents in drug makers; chem int for antihistamines (including chloropheniramine maleate); reagents (including as acid removers); int for anti-infectives including cetylpyridinium chloride (National Library of Medicine, 2024).

b. Aldosterone

Aldosterone is a steroid hormone that regulates salt and water in the body, affecting blood pressure. The salt and water content in the body must be balanced, as too much salt consumption can lead to fluid retention, which can increase blood pressure. High blood pressure is a major risk factor for heart attack, stroke and heart failure (Escher, 2009). Aldosterone is a hormone secreted by the adrenal cortex that regulates electrolyte and water balance by increasing renal sodium retention and potassium excretion (National Library of Medicine, 2024).

c. Combretastatin

Combretastatin has been shown in the laboratory to stop the blood supply to tumours. It was one of the first drugs to target blood vessels and was tested in patients (National Library of Medicine, 2024). Combretastatin has been investigated for the treatment of Anaplastic Thyroid Cancer (National Library of Medicine, 2024). Combretastatin is a stilbenoid phenol, originally isolated from the bark of the African willow Combretum caffrum, with vascular disrupting and antineoplastic activities (National Library of Medicine, 2024).

d. Cobalt

Cobalt is a Standardised Chemical Allergen. The physiological effects of cobalt are through Increased histamine release and cell-mediated immunity. Cobalt is an essential nutrient in the human diet in the form of Vitamin B12 (cyanocobalamin) (National Library of Medicine, 2024). Cobalt (Co) is a metal that has an important role in industry and the sustainable life of the modern world. Cobalt has physical and chemical properties that are very useful in various metallurgical and chemical applications. This metal is mainly used as a superalloy material in jet engines, turbines, gas catalysts, pigments, magnets, and special steels (Hendro et al., 2022). Currently, cobalt is mostly used for the manufacture of cathode material in rechargeable lithium-ion batteries (Hendro et al., 2022).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis test of methanol extract of chloroform fraction produced 2 peaks that have active compounds, namely peak 2 and peak 2. The dominant peak that has the highest percent area is peak 3 (21.55% area) by producing pyridine, aldosterone, combretastatin, and cobalt compounds. Peak 2 produces glycine compounds and peak 3 produces pyridine, aldosterone, combretastatin, and cobalt compounds.

CONCLUSION

Conclusions

1. There are secondary metabolite compounds of parijoto fruit methanol extract as evidenced by the results of phytochemical screening tests, namely alkaloids, polyphenols, quinones, saponins, molish reagent glycosides, flavonoids, and tannins.
2. There are differences in the number of secondary metabolite compounds of parijoto fruit (Medinillla speciosa Blume) in the methanol extract of ethyl acetate fraction and chloroform fraction using GC-MS. The compound results from the ethyl acetate fraction are 1,2 - benzenedicarboxylic acid and bis(2- ethylhexyl) phthalate (30.59% area), octadecanoic acid (12.49% area), cyclopentanethiol (11.40% area), hexadecanoic acid (9.14% area), 10-undecenal (8.75% area), tetracosanoic acid and eicosanoic acid (4.21% area), dimethyl sulfate and pentanoic acid (3.74% area), and 8-cyclohexadecen-1-one (1.75% area). The compound results from the chloroform fraction are pyridine, aldosterone, combretastatin, cobalt (21.55% area) and glycine (9.84% area).
3. The highest number of secondary metabolite compounds of parijoto fruit (Medinillla speciosa Blume) from methanol extract and ethyl acetate fraction and chloroform fraction using GC-MS is ethyl acetate fraction with 11 compounds.

Suggestion

Suggestions that can be given in further research are:

1. Re-examine the benefits of parijoto fruit, by conducting research on other benefits of parijoto fruit.
2. Further research of the ethyl acetate fraction and chloroform fraction should be able to make a preparation.

REFERENCES

Annona, S., Febriani, D., Mulyanti, D., & Rismawati, E. (2015). Characterisation of simplisia and ethanol extract of soursop leaves, Journal of Pharmacy, 10(2), 475-480.

Ashford, RD. (1994). Ashford's dictionary of industrial chemistry. London, UK: Wavelength Publications Ltd.

Damayanti, P. N., Luhurningtyas, F. P., & Indrayati, L. L. (2023). Determination of total phenolic and flavonoid content of ethanol extract of parijoto fruit (medinilla speciosa blume) by UV-Vis spectrophotometric method. Journal of Pharmacy, 12(1), 1-6.

Dewatisari, W. F. (2020). Comparison of chloroform and ethanol solvents on the yield of tongue-in- law (sansevieria trifasciata prain) leaf extract using maceration method. Journal of Pharmacy, 3(1), 127-132.

Eko B. M. (2015). Phytochemical screening and total flavonoid content in carica pubescens lenne & k. koch fruits in bromo, cangar, and dieng plateau areas. Journal of Phytochemical Screening, 5(2), 73-82.

Fiehn, O. (2002). Metabolomics the link between genotypes and phenotypes. Plant Molecular Biology, 48(1), 155-171.

Handayani, Wirasutisna, & Insanu. (2017). Phytochemical screening and characterisation of rose guava (Syzygium jambos Alston) leaf simplisia, Journal of Phytochemical Screening, 5(1), 22-24.

Hendro Purnomo, Shilvyanora Aprilia Rande, Rizqi Prastowo. (2022). Spatial mapping of cobalt content in laterite deposits using ordinary cokriging and inverse distance weighting methods. Journal of Science Technology & Environment. 8(1) 73-86.

Heryana, A. (2014). Research hypothesis. Yogyakarta: Eureka Education, 1.

Illing, I., Safitri, W., & Erfiana. (2017). Phytochemical test of degen fruit extract. Journal of Dynamics, 8(1), 66-84.

Lanipi, R. P., Hardia, L., Astuti, R. A., & Budianto, A. B. (2022). Phytochemical test of katuk (sauropus adrogynus (L) merr) leaf extract. Journal of Ethnopharmacy, 1(1), 1-6.

Larranaga, M.D., Lewis, R.J. Sr., Lewis, R.A. (2016). Hawley's Condensed Chemical Dictionary 16th Edition. John Wiley & Sons, Inc. Hoboken, NJ, 1027.

Leliana N. W. (2013). Thesis Test of antioxidant activity and determination of total phenolic and

flavonoid content of parijoto fruit (medinilla speciosa blume). Institutional Repository UIN Syarif Hidayatullah Jakarta.

Mahmiah, Sudjarwo, G. W., & Andriyani, F. (2017). Phytochemical screening and GC-MS analysis of hexane fraction of stem bark (Rhizophora mucronata L.) National Marine Seminar XII, 44- 51.

Maryanti, A. (2016). Isolation and characterisation of endophytic fungi from the twigs of parijoto plants (medinilla speciosa blume) and their activity test as antibacterial. Thesis, Institutional Repository UIN Syarif Hidayatullah Jakarta.

Niswah, L. (2014). Antibacterial activity test of parijoto fruit extract (medinilla speciosa blume). Pharmacy Study Programme, Faculty of Medicine and Health Sciences, State Islamic University Syarif Hidayatullah Jakarta.

Nugroho, A., & Mangkurat, U. L. (2017). Natural Materials Technology. Textbook: natural materials technology. Banjarmasin: Sains Techno Langsung Perum Korpri, Pangkalpinang.

O'Neil MJ, Heckelman PE, Dobbelaar PH, Roman KJ. (2013). Merck Index, Encyclopedia of Chemicals, Medicines, and Biologicals. 15th edition. Cambridge, UK: The Royal Society of Chemistry.

Penketh PG, Shyam K, Divo AA, Patton CL, Sartorelli AC. (1990). Methylating agents as trypanosides. Journal of medicinal chemistry, 33, 730-732.

Sarah Melinda, E. Annisaa', and W. Sasikirana. (2021). Cytotoxic potential of purified parijoto (medinilla speciosa blume) fruit extract on HeLa cervical cancer cells. Generics: Journal of Research in Pharmacy, 1(2), pp. 44 - 52.

Suratno, S. (2016). Phytochemical screening of ethanol extract of spirulina platensis microalgae with potential as antibacterial. Surya Medika Journal, 1(2), 26-33.

Syarif. (2015). Ethyl acetate extract of parijoto fruit (medinilla speciosa blume). Jakarta.

Van Hove JL, Vande Kerckhove K, Hennermann JB, Mahieu V, Declercq P, Mertens S, De Becker M, Kishnani PS, Jaeken J. (2005). Benzoate treatment and the glycine index in nonketotic hyperglycinaemia. Journal of Education Research. 28(5):651-63.