LAMPIRAN

Lampiran 1

Rendemen Ekstrak Buah Merah

1. Minyak Buah Merah

   a. Buah Merah : 6000 g
   b. Air : 7000 ml
   c. Hasil Minyak : 100 ml

   $$\% \text{ Rendemen} = \frac{\text{Hasil Minyak}}{\text{Buah Merah}} \times 100\%$$

   $$= \frac{100 \text{ ml}}{6000 \text{ g}} \times 100\%$$

   $$= 1{,}67 \ \%\ {}^{v}/_{b}$$

2. Sari Buah Merah

   a. Buah Merah : 6000 g
   b. Air : 7000 ml
   c. Hasil Sari : 3000 ml

   $$\% \text{ Rendemen} = \frac{\text{Hasil Sari}}{\text{Buah Merah}} \times 100\%$$

   $$= \frac{3000 \text{ ml}}{6000 \text{ g}} \times 100\%$$

   $$= 50 \ \%\ {}^{v}/_{b}$$

Lampiran 2

Perhitungan Fase Gerak Dalam Kromatografi Lapis Tipis (KLT)

1. Perhitungan Fase Gerak

   Fase gerak yang digunakan yaitu kloroform : asam asetat : metanol (95 : 5 : 1) dan dibuat sebanyak 10 ml.

   a. Kloroform

      Volume yang dibutuhkan untuk 10 ml = $\frac{95}{101}$ x 10 ml

      = 37,62 ml

   b. Asam asetat

      Volume yang dibutuhkan untuk 10 ml = $\frac{5}{101}$ x 10 ml

      = 1,98 ml

   c. Metanol

      Volume yang dibutuhkan untuk 10 ml = $\frac{1}{101}$ x 10 ml

      = 0,39 ml

Lampiran 3

Perhitungan Nilai Rf Dan HRf Pada Analisi KLT

- $Rf = \frac{\text{Jarak yang ditempuh sampel}}{\text{Jarak yang ditempuh pelarut}}$
- hRf = Rf x 100

a. Sampel minyak buah merah produk manual

- $Rf = \frac{7,2}{7,4} = 0,972$
- hRf = 0,972 x 100

  = 97,2

b. Sampel sari buah merah

- $Rf = \frac{7,2}{7,4} = 0,972$
- hRf = 0,972 x 100

  = 97,2

c. Sampel minyak buah merah (merk x)

- $Rf = \frac{7,1}{7,4} = 0,959$
- hRf = 0,959 x 100

  = 95,9

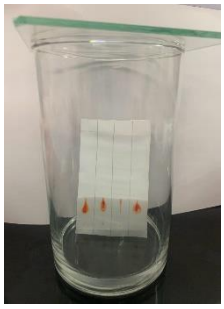
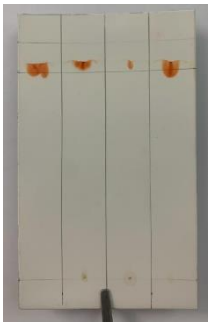
Lampiran 4

Proses Pembuatan Minyak Buah Merah

No	Gambar	Keterangan
1.		Sampel Buah Merah (*Pandanus conoideus*)
2.		Pemisahan biji dan buah dari empulur
3.		Proses Pembuatan Minyak Buah Merah
4.		Proses Perebusan Minyak Buah Merah
5.		Minyak Buah Merah

Lampiran 5

Proses Analisis Kromatografi Lapis Tipis

No	Gambar	Keterangan
1.		Menunggu hingga eluen naik
2.		Hasil Kromatografi Lapis Tipis

Lampiran 6

Sertifikat Jurnal

Lampiran 7

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Physical Characteristics And Phytochemical Screeningfrom Oil And Red Fruit Juice (*Pandanus Conoideus* L.)

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ABSTRACT

Red Fruit Oil contains several active compounds that are beneficial for the human body such are tocopherol, betacarotene and some types of fatty acids such as oleic acid, linoleic acid, linolenic acid and decanoic. The purpose of this study was to determine the content of secondary metabolites in oil and red fruit juice (Pandanus conoideus). This research method uses qualitative data research methods, namely from the results of analysis of the content of secondary metabolites of oil and Red Fruit juice (Pandanus conoideus) consisting of alkaloids, flavonoids, terpenoids, saponins and tannins, as well as thin-layer chromatography. The results of the study showed that phytochemical screening tests of red fruit oil and red fruit juice hand products gave the same results as red fruit oil (brand x) on the market, that the three samples contained several secondary metabolite compounds namely flavonoids, tannins, and terpenoids

Keywords: *Red Fruit, Phytochemical Screening, Thin Layer Chromatography.*

1. INTRODUCTION

The red fruit plant (*Pandanus conoideus* L.) is a plant in the Pandanus family. Red fruit (*Pandanus conoideus* L.) is a type of pandan plant typical of Papua. This plant is often found in Papua, Papua New Guinea, and has begun to be planted sporadically in several areas such as Maluku, Sulawesi, Kalimantan, Java and Sumatra.[1] The red fruit plant (*Pandanus conoideus* L.) is an endemic species to Papua which has the potential to become the only phytopharmaceutical in Indonesia. By local Papuan residents, this plant, which belongs to the Pandanaceae family, has been used as traditional medicine. People have used red fruit juice made from the flesh of the fruit to treat various degenerative conditions, including diabetes, gout, hypertension, stroke, and cancer.[2]

People outside Papua know and consume red fruit oil that has been processed and produced on a factory scale. They consume red fruit oil because it can help treat several diseases and as a *food supplement*.[3] Red fruit oil contains active compounds that are beneficial for the human body. These active compounds include tocopherol, beta-carotene, and several types of fatty acids such as oleic acid,

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linoleic acid, linolenic acid, and decanoate. Tocopherol is efficacious as an antioxidant so it can strengthen the immune system and ward off free radicals. Beta-carotene functions as asupplier of vitamin A.[4]

Red fruit juice contains large amounts of antioxidant compounds, namely tocopherol (11,000 ppm), beta-carotene (700 ppm), and carotenoids (12,000 ppm) as well as antioxidant compounds.[5] Red fruit juice has the potential to inhibit the growth of lymphocyte-producing cells and the spread of cancer-causing cells, thereby increasing the risk of death from this disease in Indonesia.[5] Consuming beta-carotene regularly allows the body to manage disease symptoms. The cells in question will relieve the symptoms of cancer cells by neutralizing the carcinogens that cause disease at the root. However, in-vivo experiments using Sparague Dawley rats show that red fruit can be effective for optimal tissue growth and maintenance.[5] Various types of plants contain secondary metabolite compounds, such as alkaloids, flavonoids, steroids, terpenoids, saponins and others, which are bioactive substances related to the chemical content in plants, so that some plants can be used as medicinal ingredients.[6]

Bioactive compounds are active compounds that are responsible for the ongoing metabolic reactions that benefit health.[6] Bioactive component testing can be done using phytochemical testing. Phytochemical testing aims to determine the characteristics of active compounds that can be utilized and active compounds that cause toxic effects by means of crude extracts. Phytochemical screening is a simple, fast and highly selective method, which can be used to identify groups of compounds and determine the presence of biologically active compounds distributed in plant tissue.[6]

This research is part of the initial stages of research that has previouslu been carried out. Researchers conducted experiments by directly making oil and juice obtained from red fruit, these raw materials weee obtained directly from the Papua region, The oil and juice that have been made are then tested to determine the content of secondary metabolite compounds contained therein.

2. EXPERIMENTAL

2.1. Tools and materials

The tools used in this research were a volume pipette, analytical balance, measuring cup, steam cup, flannel cloth, stirring rod, glass beaker, drop pipette, glass funnel, test tube, gloves, mask, 60 mesh sieve, oven, microscope , 20 exchange cups, TLC chamber, silica plate, glass object, Degg glass, test tube, test tube rack, flannel cloth, filter paper, bath, tripod, bunsen.

The materials used in this research were samples in the form of red fruit, distilled water, gallic acid, Folin-Ciocalteau reagent, distilled water, acetic acid, FeCl3, chloroform, acetic acid, methanol, Na2CO3, 96% ethanol, 70% ethanol, ammonia, HCl (hydrochloric acid) concentrated, anhydrous acetic acid, concentrated sulfuric acid, sulfuric acid (H2SO4) 2N, ferric chloride (FeCl3) 1%, chloroform, methanol, hexane, butanol, Mayer's reagent, Dragendroft's reagent, Wagner's reagent, Mg metal, ethyl acetate, potassium iodide, nitric acid, bismuth nitrate.

2.2. Research procedure

1.Macroscopic Idntification Test

Macroscopic testing is used to see the physical appearance of preparations by examining the shape, color, smell and taste of the samples studied.

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Indonesian Journal of Chemical Science and Technology (IJCST-UNIMED) , 2024, Volume 07, No 1, pp 82-88

2.Making Red Fruit Oil

According to Limbongan and Malik (2009) in (Subrata et al., 2019) the steps for making red fruit oil are:[7]

a. choose truly ripe fruit;
b. the fruit is split open and the pith is removed;
c. fruit flesh cut into pieces and washed clean;
d. fruit flesh is steamed for 1 hour to 1 hour 30 minutes until soft;
e. fruit removed and cooled;
f. add a little water then knead and squeeze until it becomes a paste;
g. the paste is then filtered to separate the seed pulp from the paste;
h. pasta cooked 4 to 5 hours until boiling;
i. the paste is left on the fire for 10 minutes until black oil appears on the surface;
j. Remove the boiled pasta then let it rest for 1 hour;
k. scoop the oil slowly using a spoon into a transparent container;
l. Leave for 2 hours until the oil separates from the water and pasta.
m. The steps for making red fruit oil are repeated several times until there is no more water under the oil layer. Water can also be removed by heating the oil at 95 to 100°C for 2 to 3 minutes until no more water bubbles are visible. The final result is fruit juice or called red fruit oil and is then cooled.[7]

3.Phytochemical Screening

1. Alkaloids
   Alkaloids can be made by taking 1 ml of extract. Then add 1 ml of chloroform, 1 ml of ammonia, heat and filter. The filtrate obtained was divided into three parts, 2N sulfuric acid was added to each part. Mayer's reagent was added to the 1st filtrate, Wagner's reagent was added to the 2nd filtrate and Dragendrof's reagent was added to the 3rd filtrate. Positive results indicate that a white precipitate was formed in Mayer's reagent, a brown precipitate was formed in Wagner's reagent and a red precipitate was formed in Dragendrof's reagent.[8]
2. Flavonoid
   A 1 ml sample was added to 3 ml of 70% ethanol then shaken, heated and shaken again. Filter the filtrate. The filtrate obtained was added with 0.1 gram Mg and 2 drops of concentrated HCl. A positive result shows a red color in the ethanol layer.[8]
3. Terpenoid/streroid
   Add 1 mL of oil to 3 mL of 70% ethanol, 2 mL of concentrated sulfuric acid and 2 mL of anhydrous acetic acid. Positive results show a purple to blue color change for steroids and the formation of a brownish red color on the surface indicates the presence of triterpenes.[8]
4. Saponin
   1 ml of oil, add 10 ml of distilled water then heat. The filtrate obtained was shaken and left for 15 minutes, then added 2 drops of 2N HCL. A positive result will indicate stable foam.[8]
5. Tannin
   1 ml of oil, add 20 ml of distilled water, heat then filter the filtrate. The filtrate obtained was added with 2-3 drops of 1% $FeCl_3$. A positive result shows a greenish brown or blackish blue.[8]

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4. Thin layer chromatography

First prepare the tools and materials. The silica gel coated TLC plate that will be used is first oven at 4 °C for 3 minutes to reduce the water content in the TLC plate. Furthermore, after being oven-treated, the TLC plate is given a top and bottom border of 1 cm each to make it easier to spot and know the distance the solvent has traveled, making it easier to calculate Rf. Then make the mobile phase by taking chloroform: ethyl acetate: n-butanol: formic acid (5: 2: 2: 1) making 10 ml. Insert it into the chamber and saturate it by inserting filter paper into the chamber and covering it with a glass cover. When it is saturated, the eluent will come out through the filter paper during elution, the silica gel will absorb the mobile phase. The next process is inserting the TLC plate on which the sample has previously been stained into the saturated chamber. In this process the mobile phase will move up through the silica gel granules and the movement will be followed by the identified compound. After the elution process, the silica gel plate is marked by raising the eluent to the upper limit line then lifting the TLC plate then drying it in the air after which the appearance of the stain is seen using 254 and 366 nm UV light. A good eluent is one that can separate compounds in large quantities, indicated by the appearance of a stain. The conditions for good spots are that they have no tails and the distance between one spot and another is clear. The next process analyzes the Rf and compares it with the theoretical standard Rf value.[9]

3. RESULTS AND DISCUSSION

3.1 Physical characteristics of fruit, oil and red fruit juice

The red fruit used was purchased directly from farmers in Manokwari Papua. The fruit selected is seen from the optimal level of ripeness, with the harvest criteria being that the fruit pulp is full (*nasty*), which is marked by a change in color of the fruit from green, pink, pale red when the fruit is young and changes to blackish red when the fruit is ripe.[10]

The following is a description of the physical characteristics of the red fruit samples used in the research:

Tabel 1. Macroscopic characteristics of Red Fruit

Form	Color	Size		Image
		Long (cm)	Weight (kg)	
Triangular cylinder (tapered)	Red	50 - 73	2,7 – 6,2	

(source: primary research data)

The results of the physical characteristics of the whole fruit in the red fruit in this study can be seen from its shape, which is a tapering triangular cylinder, from the rounded base extending to the middle, expanding or shrinking to the tip, which is different between clones. Several researchers describe the red fruit as triangular and cylindrical in shape, with a blunt or sharp tip, and a cardiac base.[3]

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Of the 2 (two) variations of red fruit, the sizes obtained were an average length ranging from 50-73 cm and a weight of 2.7-6.2 kg. Murtiningrum et al. (2012) grouped red fruit based on size, namely long-sized fruit (>50cm), medium-sized fruit (49-53cm) and short-sized fruit (>35cm). Meanwhile, based on fruit weight, Hadad et al. (2016) grouped the weight of small red fruit in the range of 2-7 kg and large fruit in the range of 5-10 kg.[10]

Tabel 2. Macroscopic characteristics of red fruit oil and juice.

No	Results	Form	Smell	Color	Flavor
1	Oil	Thick liquid	Typical	Red	Tasteless
2	Essence	Semi solid	Typical	Red	Tasteless

(source: primary research data)

3.2 Phytochemical Screening

Tabel 3. Phytochemical Screening

No	Compound Test	Reactor	References	Test results		
				A	B	C
1.	Alkaloids	Mayer	Yellowish White Precipitate	-	-	-
		Wagner	Red Precipitate	+	-	+
		Dragendroff	Brown Precipitate	-	-	-
2.	Flavonoids	Mg + Concentrated HCL	Red	+	+	+
3.	Triterpenoids	Ethanol 70%+ H2SO4 concentrated + acetic acid anhydrous	Red	+	+	+
4.	Saponins	Aquadest + HCL 2N	Foamy	-	-	-
5.	Tannins	FeCl3	Greenish Brown/Blackish Blue	+	+	+

(source: primary research data)

Information :
A : red fruit oil
B : red fruit juice
C : red fruit oil (brand x) on the market
(+) : contains the tested compound
(-) : does not contain the tested compounds

Phytochemical screening is the initial stage in phytochemical research which is aimed at providing an overview of a class of compounds found in the plant being studied. (kristiani 2008).[11] Screening Phytochemistry in this research is aimed at determining the metabolite content secondary in red fruit oil. Fruit oil phytochemical screening test results red and red fruit juice manual products show the same results as red fruit oil (brand X) on the market, that all three

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contains several secondary metabolite compounds except alkaloid compounds Mayer, Dragendorf, and Saponin as shown in **Table 3**.This is in accordance with what has been stated by Asrianto, et al (2022) meyer's saponin and alkaloid compounds were not identified in all solvents used for red fruit extract, but red fruit ethanol extract contained tannin, flavonoid, steroid, triterpenoid and alkaloid compounds, showing positive results. Meanwhile, the phytochemical test of red fruit using methanol solvent was positive for containing tannins, flavonoids, steroids and alkaloids. Apart from that, phytochemical testing of red fruit using hexane as a solvent gave positive results for the content of steroid and terpenoid compounds.[12]

3.3 Thin Layer Chromatography

The following is data from observations of layer chromatography identification thin on oil and red fruit juice:

Tabel 4. Thin Layer Chromatography Results

Sampel	Sampel		Standar (Akhmad, 2015)	
	Rf	HRf	Rf	HRf
A	0,97	97		
B	0,97	97	0,90	90
C	0,95	95		

(source: primary research data)

Based on the data table above, it is known that for samples A, B, and C, Rf values were obtained which were close to the standard Rf value, namely 0,97; 0,97 and 0,95 with yellow-orange colored spots respectively where the spots indicate the presence of flavonoid compounds. A sample is declared to contain flavonoid compounds if the spots are yellow, with an Rf value = 0,9.[13] Where flavonoids are one of the phenolic group compounds, so this shows that the phenolic compounds produced are good.

4. CONCLUSION

Based on the research results above, it can be concluded that the results of the phytochemical screening test for red fruit oil and red fruit juice from manual products show the same results as red fruit oil (brand x) circulating on the market. It is known that all three contain several secondary metabolite compounds, namely flavonoids, tannins and terpenoids.

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