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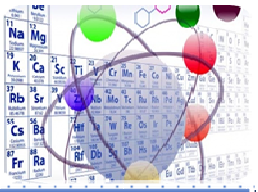

The Properties of Ethyl Acetate Extract of Frankincense (Styrax benzoin) Using the Maceration Method

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ABSTRACT

This study aims to determine the standardization of ethyl acetate extract of frankincense gum using the maceration method. The method used in this study was to standardize the ethyl acetate extract of frankincense using specific and non-specific parameters. The results of standardization with specific parameters showed that the ethyl acetate extract of frankincense latex was blackish brown in color and had a distinctive odor of frankincense. It contained 86.5% soluble compound in ethanol. The results of standardization with non-specific parameters showed a specific gravity of 0.8213 g/ml and an average moisture content of 1.71%. The chemical components contained are Benzoic acid; Vanillin; trans-Cinnamic acid; Phenol, 2-methoxy-4-propyl-; 2-Propanone, 1-(4) -hydroxy-3-methoxyphenyl)-; Benzoic acid, phenylmethyl ester; (Z)-Cinnamyl benzoate; 2-Propenoic acid, 3-phenyl-,phenylmethyl ester, (E) -; and Cinnamyl cinnamate.

Keywords: Styrax benzoin, standardization, extracts, chemical component, GC-MS

1. INTRODUCTION

Frankincense (Styrax benzoin) is one of Indonesia's unique natural resources which produces frankincense sap. Frankincense trees are spread in various countries such as Thailand, Malaysia, Laos and Indonesia.[1] The main constituents of the chemical components of the sap of the incense tree are cinnamic acid and benzoic acid. Sumatran frankincense also contains many phenylpropanoid derivatives such as benzaldehyde, vanillin, styrol, phenylpropyl cinnamic, also contains benzoresinol esters, coniferyl alcohol esters from cinnamic acid and benzoic acid.[2] Frankincense sap is used as a fragrance and is also used as an antioxidant in the cosmetic industry and as a flavor enhancer in the food industry.[3] Frankincense is also used traditionally to inhibit microbial growth and to treat rheumatic diseases.[4] Because of the many potentials of frankincense gum extract, it is necessary to standardize frankincense gum extract.

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Standardization is a series of parameters, procedures and ways of measuring the results of which are related elements such as a quality paradigm that meets standards and guarantees product stability. Standardization is carried out so that the plants to be used as raw materials for traditional medicines are of good quality according to the requirements.[5] Standardization includes 2 important aspects, namely aspects of specific parameters and non-specific parameters. Aspects of specific parameters are focused on active compounds that are responsible for providing pharmacological effects which include, organoleptic, compounds dissolved in certain solvents, chemical content tests. The non-specific parameters include drying shrinkage, specific gravity, ash content, moisture content, residual organic solvents, microbial contamination, and heavy metal contamination.[6] In this research, standardization of ethyl acetate extract of frankincense gum was carried out by testing specific parameters and non-specific parameters. Specific parameters which include identity and organoleptic extract, compounds dissolved in ethanol solvent and extract phytochemical screening for non-specific parameters which include determination of specific gravity and determination of water content.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The materials used in this study were 80 mesh incense resin powder from the Parsoburan area, ethyl acetate, ethanol, propylene glycol (PG), tween 80, distilled water, magnesium powder, concentrated HCl, dragendorrf reagent, 2N HCl, $FeCl_3$, filter paper and aluminium foil.

2.2. Research Procedure

2.2.1. Extraction

As much as 250 g of frankincense gum powder was extracted by maceration or immersion using ethyl acetate distillate with a ratio of 1:2 and carried out in a glass jar for 3x24 hours while stirring occasionally. Then the solution was filtered using filter paper with the help of a bunchner and a vacuum pump. After that, all the filtrate obtained was concentrated using a rotary evaporator until the sample became thick.

2.2.2. Specific Standardization.

Identity and Organoleptic Extract

The identity of the extract includes nomenclature, the Latin name of the plant, the part of the plant used and the Indonesian name of the plant. The organoleptic of the extract includes observation of shape, color and smell.

Ethanol Soluble Extractive Content

A total of 5 grams of extract was dissolved with 100 ml of 96% ethanol in a measuring flask and shaken. After 24 hours, it was filtered quickly and 20 ml of the filtrate was evaporated to dryness at 105°C to a constant weight. The concentration in percent of the compound dissolved in ethanol was calculated by the initial extract weight.[7]

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Phytochemical Screening

Identification of flavonoids was carried out by mixing 1 gram of extract with 5 ml of ethanol, shaking, heating and shaking again then filtering. Then 0.2 g of Mg powder and 3 drops of HCl were added to each filtrate. If there is a color change from orange to purple red, it indicates the presence of flavonoids.[5] Alkaloid compounds were identified using the Dragendorff reagent, the brick red precipitate that formed showed a positive test for alkaloids. Condensed extract of frankincense sap as much as 1 gram is dissolved with distilled water that has been heated. The solution was then shaken vertically for 10 seconds and allowed to stand for 10 seconds. The foam that appears with a height of 1-10 cm is added 1 drop of 2N HCl, the formation of stable foam after adding acid indicates a positive saponin test. Identification of tannin compounds by adding 1% $FeCl_3$ to frankincense latex extract in a test tube, the test will be positive if the color changes to black-green[3]. Phenol identification was carried out by adding 0.5 g of sungkai stem bark extract to 5 ml of ethanol, filtered, and added with 1 ml of iron (III) chloride ($FeCl_3$). If a purple-blue color is formed, the positive extract contains phenolic compounds.[8]

2.2.3. Non Specific Standardization

Density of extract

The specific gravity of the extract was determined based on the dilution results of 5% liquid extract using a pycnometer. A clean, dry (W_0) pycnometer that has been calibrated by setting the weight of the pycnometer is used. Fill the pycnometer with liquid extract and give it a temperature of 25°C, remove excess liquid extract and weight (W_1).[9]

Water Content

Weigh a porcelain cup, then dry it in an oven at 105°C for 30 minutes, then place it in a desiccator for 30 minutes. After that, the porcelain cup was weighed again. Then 10 g of sample was put into a porcelain cup, then heated using an oven at 105°C for 30 minutes, then placed in a desiccator for 30 minutes and weighed again. Calculated percentage of water content.

2.2.4. Chemical Component Analysis

Analysis of the ethyl acetate extract of frankincense was carried out using Gas Chromatography-Mass Spectrometry (GC-MS) using the GCMS 7890B system, with HP1 column (30 m x 250 µm x 0.25 µm).

3. RESULTS AND DISCUSSION

3.1. Extraction results

The extraction results obtained a viscous extract of frankincense gum which was blackish brown in color and the weight of the extract obtained was 182.158 gr with a % yield of 72.86%.

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3.2. Specific Standardization of Ethyl Acetate Extract of Frankincense (Styrax benzoin)

The results of testing the specific parameters of identity and organoleptic extract can be seen in the following table.

Table 1. Extract identity and organoleptic parameters

Parameter	Results
Extract Identity:	
Extract name	*Styrax benzoine Extractum*
Latin name	*Styrax benzoine* Dryand
Plant parts	*Styrax benzoin Gum*
Indonesian Names of Plants	Kemenyan durame
Organoleptic extract:	
Form	Viscous extract
Color	Dark brown
Smell	Frankincense smell

The results obtained from the determination of compounds that dissolve in ethanol are as much as 86.5%. The concentration results obtained are high, based on SNI Frankincense alcohol solubility > 75%.[10] High yields indicate that the extract has higher levels of semi-polar compounds. This is because the solvent used in the maceration process is ethyl acetate which is semi-polar and has a polarity similar to ethanol. Determination of the content of compounds that dissolve in ethanol is a general estimate of the number of compounds that are semipolar (dissolved in ethanol). Determination of ethanol-soluble compounds does not directly affect the pharmacological effects of active compounds in extracts.[6]

The results of the phytochemical screening test for the ethyl acetate extract of frankincense can be seen in the following table

Table 2. Parameters of ethanol soluble extractive content

Chemical Substance	Reagent	Results
Flavonoid	Mg and HCl	+
Alkaloid	Dragendorff	+
Saponin	Aquadest and HCl	+
Fenol	$FeCl_3$	+
Tanin	$FeCl_3$ 1%	+

Identification of the class of chemical compounds in the ethyl acetate extract of frankincense was carried out using chemical reagents. Identification of the class of chemical compounds contained in the ethyl acetate extract of frankincense latex tested positive for flavonoids, alkaloids, saponins, tannins and phenols (Table 2.). The results of the identification test showed the presence of flavonoids which was indicated by the

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formation of a reddish-orange color. Testing the alkaloid content using the dragendorff reagent showed positive results for the presence of alkaloid compounds with the formation of a brick red precipitate. In the identification of saponins, ethyl acetate extract of frankincense gum showed positive results with the appearance of stable foam as high as 1 cm. This is indicated by the color change that occurs when adding 1% $FeCl_3$ solution, namely moss green.

3.3. Non Specific Standardization of Ethyl Acetate Extract of Frankincense (Styrax benzoin)

In the test to determine the density of extracts carried out using a pycnometer. The pycnometer must be cleaned and dried beforehand so that there is not the slightest drop of water in it. This aims to obtain the empty weight of the pycnometer. If there is still water inside, it will affect the results. The extract used is an extract that has previously been diluted to a 5% liquid extract. The results obtained in this measurement were 0.8213 g/ml for a 5% dilution of the ethyl acetate extract of frankincense gum. With this it can be described the amount of mass per unit volume to provide a boundary between liquid extracts and viscous extracts, besides that density is also related to how to know the purity of a substance which is determined by its density.[9]

Determination of water content is carried out to determine the remaining water contained in the extract which will then guarantee the quality and storage of the extract. The water content can determine the stability of the extract and subsequent dosage forms.[6] The test results obtained for the water content in the ethyl acetate extract of incense sap were an average of 1.71%. This shows that the ethyl acetate of frankincense has good quality because the water content contained in the extract does not exceed 10%. The high water content in the extract can result in the rapid growth of fungi in the extract.[11]

3.4. The Results of The GC-MS Analysis

Following are the results of the GC-MS chromatogram analysis of the ethyl acetate extract of frankincense gum.

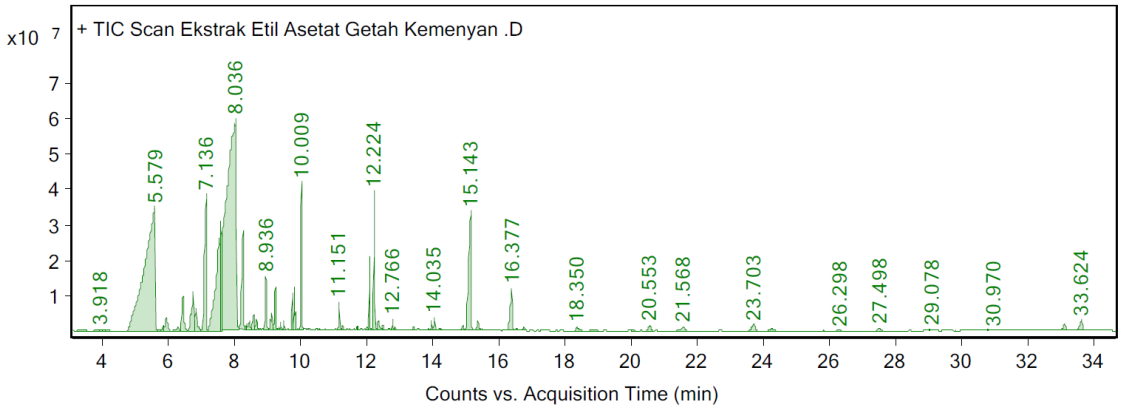

Figure 1. GC-MS Chromatogram Results of Frankincense Ethyl Acetate Extract

Analysis using GC-MS was carried out to determine the chemical components contained in the ethyl acetate extract of frankincense latex. From the results of the GC-MS analysis of ethyl acetate extract of

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frankincense gum, there are components detected in GC-MS consisting of many compounds with varying Rt (retention time) and % area. Components that have the largest percent area in GC-MS are presented in Table 3. The table shows the largest components in the frankincense latex extract, namely cinnamic acid, benzoic acid and vanillin.

Table 3. Results of GC-MS Analysis of Frankincense Ethyl Acetate Extract

Peak	RT	%Area	Chemical Components
1	5.579	64.34	*Benzoic acid*
2	7.136	14.49	*Vanillin*
3	7.517	20.15	*trans-Cinnamic acid*
4	7.575	8.06	*Phenol, 2-methoxy-4-propyl-*
5	8.036	100	*trans-Cinnamic acid*
6	8.244	8.94	*2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-*
7	10.009	8.87	*Benzoic acid, phenylmethyl ester*
8	12.074	2.58	*(Z)-Cinnamyl benzoate*
9	12.224	6.65	*2-Propenoic acid, 3-phenyl-, phenylmethyl ester, (E)-*
10	15.143	15.58	*Cinnamyl cinnamate*

4. CONCLUSION

The results of specific parameters include: Organoleptic incense in the form of a viscous blackish brown extract with a distinctive odor of frankincense, determination of the soluble compound content in ethanol as much as 86.5%, the extract contains flavonoids, alkaloids, saponins, tannins and phenols. The results of non-specific parameters include: density of 0.8213 g/ml and the results of determining the water content is an average of 1.71%. From the results of the GC-MS analysis of ethyl acetate extract of frankincense gum, largest components in the frankincense latex extract, namely cinnamic acid, benzoic acid and vanillin.

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