Isolation of Bioactive Flavonoid Compounds from the Sap of Sumatran Frankincense (*Styrax benzoin*) which have Potential as Medicinal Raw Materials

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Abstract. The flavonoid group as natural polyphenolic compounds produced by plants needs to be explored because they have medicinal properties. Sumatran Frankincense (*Styrax benzoin*) is known as a plant that produces incense resin containing bioactive substances which have been used as medicinal ingredients in traditional and modern medicine. These bioactive compounds from Sumatran forest plants need to be revealed to increase the economic value of non-timber forest products. The research aims to isolate flavonoids from Frankincense resin as a strategy to confirm the potential of Sumatran Frankincense as a source of bioactive compounds for medicinal raw materials. The research stages include isolating and identifying flavonoid compounds contained in frankincense sap. The research results demonstrated success in isolating bioactive flavonoids from frankincense sap using various types of solvents. Isolates have been identified qualitatively to confirm the presence of bioactive compounds in the flavonoid group. The pure isolate is in the form of a yellowish-white crystalline powder and has an aroma, soluble in alcohol solvents, soluble in hot water, and difficult to dissolve in cold water. boiling point 285 ^{o}C and melting point 81 ^{o}C. Initial analysis to identify the presence of flavonoids in the frankincense sap isolate was carried out using FTIR and showed the functional groups of the flavonoids contained in the frankincense sap.

Keywords: Flavonoids; Sumatran Frankincense; Styrax benzoin; Bioactive compounds; Medicinal compounds.

1 Introduction

The need for flavonoid compounds with medicinal properties is increasing following their use, especially as medicinal ingredients for various types of applications such as anticancer, antioxidant, anti-inflammatory and antiviral [1,2]. The flavonoid group as phytochemicals

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contained in several plants are polyphenols (aromatic alcohols) which play a role in reducing the risk of chronic diseases such as cardiovascular disease and other diseases [3]. One of the plants that contains flavonoids is Sumatran Frankincense (*Styrax benzoin*), which contains bark sap which is known as frankincense sap. Frankincense resin is one of the forest products from several districts in North Sumatra, Indonesia [4]. Thus, studies on the potential of Sumatran Frankincense as a bioactive source, especially the flavonoid group, need to be intensified to reveal the content of chemical compounds with medicinal properties, thereby increasing the economic potential of North Sumatra's forest plants [5].

The problem faced is that scientific information regarding the content of bioactive compounds in the flavonoid class of Sumatran Frankincense has not been revealed, even though Frankincense sap has long been used as a medicinal mixture to cure various traditional and modern diseases. The fact is that frankincense gum in North Sumatra, Indonesia is generally still sold in the form of raw materials (frankincense sap), and chemical compounds in the form of isolates have not been carried out to obtain bioactive compounds from pharmaceutical and industrial raw materials [6]. As a result, the economic value of frankincense resin is very low when compared to the composition of the chemical compounds of the medicinal ingredients contained in the sap. A strategy must be carried out to overcome the problems mentioned above through intensive studies on the potential of frankincense sap, including through the isolation of bioactive compounds contained in frankincense sap, with the target of isolating and identifying flavonoid compounds that have high economic value and medicinal properties. The success of the bioactive isolation technique to obtain pure flavonoids will be able to increase the economic value of frankincense resin as a producer of bioactive compounds, provide an overview of the names of the chemical compounds of medicinal raw materials, and will indirectly increase the selling price of frankincense sap and will improve the welfare of farmers who live near forests [7]. This success also ensures that the supply of flavonoids for pharmaceutical and industrial purposes is met from local components.

Frankincense resin has long been used for various purposes such as religious ceremonies, medicinal ingredients, food preparations and cosmetics. Frankincense has been known as a traditional and modern medicinal mixture to cure various types of diseases because its bioactive content is effective for special treatment. Various studies on the potential of frankincense resin have been carried out to utilize frankincense, including as a raw material in the beauty, perfumery, pharmaceutical and industrial sectors [8]. The bioactive compounds contained in frankincense have been used as anti-inflammatory, antioxidant and antibacterial in various treatments [9]. Several chemical compounds in frankincense sap include cinnamic acid, benzoic acid, styracin, coniferyl benzoate, benziresinol, resinotanmol and vanillin [10]. Some of these compounds are polyphenols in the flavonoid group. The efficacy of flavonoids has the effect of stimulating the interaction of the enzymatic system which influences the healing effect in certain diseases related to viruses, bacteria and enzymatic so that it has the potential to be used as a medicinal mixture. and anti-bacterial so it can be used as a therapeutic against influenza viruses, hepatitis C viruses, and Escherichia coli [11,12]. Flavonoid compounds are also known to have anti-viral and anti-cancer properties because they function to influence the induction of apoptosis, inhibition of the proteasome, induction of differentiation, induction of cell cycle arrest, receptor interactions or interactions with carcinogens [13]. This fact encouraged researchers to isolate flavonoid compounds from the sap of Sumatran Frankincense. This research aims to isolate bioactive compounds of the flavonoid group from the sap of Sumatran Frankincense to reveal the potential of the sap as a source of bioactive medicinal raw materials.

The focus of the research is the isolation and identification of flavonoid compounds that have medicinal properties to be used as pharmaceutical and industrial raw materials.

2 Research Method

Research methods include instrumentation, materials and chemicals, and research procedures. The equipment used is a UV VIS spectrophotometer and Fourier Transform Infrared Spectroscopy (FT-IR), rotary evaporator, UV lamp and extractor.

The sample used includes incense resin which is harvested directly from selected mother plants. Chemical compounds including Methanol, Ethyl-acetate, n-hexane, Silica Gel 60 (0.063-0.200 mm), FeCl3, Acetic anhydrous, HCl, Dragendorf reagent, Chloroform, silica gel 60 F254 were all obtained from E. Merck. Standard flavonoids is used for quantitative analysis

Procedures include sample preparation, isolation and extraction, identification and confirmation of bioactive compounds. Frankincense resin is obtained from very good quality mother plants that have been identified in previous research [14]. Frankincense sap samples were tapped, harvested, dried and ground, and stored in a desiccator before being used as raw material in the bioactive compound isolation stage.

Flavonoid isolation is carried out through maceration using several types of solvents followed by extraction and fractionation. Powder samples (approximately 100 grams) were macerated using solvents with different polarities, namely n-hexane, ethyl acetate, and methanol (ratio 2:1), each for 24 hours with 3 repetitions. The next stage, the extract (from 3 types of solvents) was filtered and concentrated using an evaporator, identified qualitatively using 5% $FeCl_3$ reagent. Solutions that are positive for flavonoid compounds are then extracted, fractionated, purified, analyzed and identified. Chromatographic separation was carried out using silica gel 60F254 to obtain the best eluent composition, then identified (UV λ254 nm). The fractionation process is carried out using a column, and the resulting compound is purified and crystallized. Identification of flavonoid compounds was carried out using FT-IR to track markers of the functional groups and chemical bonds of the chemical compounds contained in the isolates. Quantitative analysis was carried out using a UV-VIS spectrophotometer using a standard solution of flavonoids dissolved in alcohol-water and measured at λ348 nm.[5].

3 Results

3.1 Isolate Flavonoid Compounds

Isolates of flavonoid compounds obtained from benzoin resin using various types of solvents have been obtained as shown in Figure 1. The results of qualitative analysis show that the highest flavonoid content was obtained in extracts macerated using a mixture of chloroform-methanol solvents (9:1). Flavonoid compounds have been identified, and from analysis the purity level of the isolate is known (85%). The isolated flavonoids are in the form of white to yellowish crystalline powder with a scented odor. Flavonoid isolates were then identified using FTIR and UV-Vis.

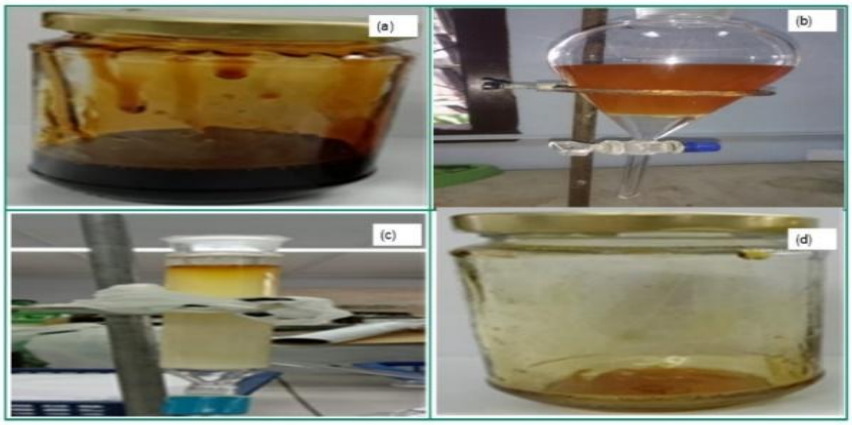

Figure 1. Stages of isolation, extraction, fractionation and purification of flavonoid compounds from Sumatran Frankincense sap: (a) Crude isolate, (b) Extraction of flavonoids, (c) Fractionation of flavonoids, (d) Flavonoid compounds.

3.2 Qualitative Analysis of Frankincense Gum Isolates

Qualitative analysis was carried out on the crude isolate compounds to confirm the presence of flavonoids as summarized in Table 1. The results of the qualitative analysis confirmed that the flavonoid isolates still contained bioactive compounds in the alkaloids, terpenoids and tannins group so they needed to be purified. The bioactive isolate is then fractionated using column chromatography to separate flavonoids from other groups of compounds. The fractionation results succeeded in purifying flavonoids which were ready for further analysis. The results of chemical analysis show that chemical compounds have chemical properties, namely soluble in alcohol solvents, soluble in hot water, and difficult to dissolve in cold water. Its physical properties are that it has a boiling point of 285 ^{o}C and a melting point of 81 ^{o}C.

Table 1. Qualitative test of Sumatran Frankincense flavonoid isolates separated using various types of solvents.

No	Class of Chemical Compounds	Qualitative analysis reagent	Extraction using various solvents (Extract)		
			Methanol	Ethyl Acetate	*n*-Hexane
1	Alkaloid group	Dragendorf	++	+++	++
2	Flavonoid group	$FeCl_3$ (5%)	++	+++	-
3	Saponin group	Aquadest & HCl	-	-	-
4	Terpenoid group	Acetic anhydrate	++	++	-
5	Tannins	$FeCl_3$ (1%)	-	++	-

3.3 Identification of Flavonoids in Sumatran Frankincense Isolates

Further identification has been carried out to confirm the presence of flavonoids in the pure isolate of frankincense gum as shown in Figure 2. The FT-IR spectrum shows the main functional groups as markers for the presence of flavonoids, and a description of the absorption peak markers for bioactive compounds is summarized in Table 2. Several groups and chemical bonds such as O-H, C-H, C=O, C=C, and C=C-H were found in the flavonoid isolates, which originate from the aldehyde functional group, hydroxyl, and ether.

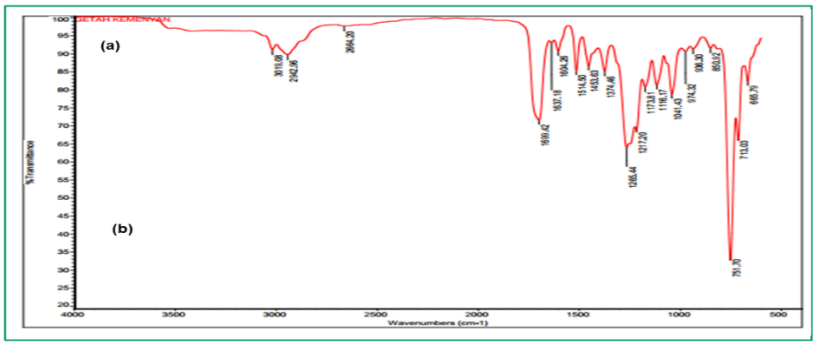

Figure 2. FTIR spectrum to identify flavonoid functional groups in Sumatran Frankincense isolates.

Table 2. Summary of functional group markers and chemical bonds in the FT-IR spectrum of pure isolates of Frankincense Gum.

Functional group markers and chemical bonds	Absorption for Flavonoids (cm^{-1})	Indicators and predictors
O-H	3400.12 and 3019.08	Positive O-H
C-H	2930.57 and 2942.96	Presence of C=H aromatic
C=O	1712.93 and 1699.42	Presence of C=O
C=C	1513.96 and 1514.50	Presence of C=C
C=C-H	711.34 and n 751.70	Presence of C=C-H

4 Discussion

Isolation of flavonoid compounds was successfully carried out using a solvent mixture of chloroform-methanol, producing a concentrated isolate of blackish brown color. Fractionation and purification of flavonoid compounds was successfully carried out using thin layer chromatography and column chromatography (Silica gel) using chloroform-methanol (9:1)

eluent to produce a flavonoid fraction with a purity of 85%. The flavonoid fraction has been identified qualitatively and quantitatively, and then recrystallized to produce a chemical compound in the form of a yellowish-white crystalline powder, accompanied by an aroma [15]. Data from analysis based on the chemical and physical properties of the isolates show that chemical compounds are close to and dominant as the characteristics of the flavonoid group [16].

Further analysis for identification and confirmation of flavonoid bioactive compounds is based on functional group markers and chemical bonds obtained from the FT-IR spectrum. Absorption data is predominantly used as a prediction for the presence of certain functional groups and chemical bonds, becoming a marker indicator for the flavonoids contained in benzoin resin [17,18]. Identification using the FT-IR absorption spectrum is still an initial prediction of flavonoid compounds and is not used to confirm the presence of compounds belonging to the flavonoid group. At this stage, difficulties are still experienced in the flavonoid isolation protocol because it is still difficult to produce pure flavonoids so further research is needed to produce flavonoid class compounds in a pure state for use in confirmatory analysis of the presence of single bioactive compounds for medicinal raw materials [19]. However, this approach is sufficient to state that frankincense sap contains flavonoids as bioactive compounds with medicinal properties.

5 Conclusion

Isolation of bioactive flavonoid compounds with medicinal properties from frankincense resin was successfully carried out through the stages of maceration, isolation, extraction, fractionation, purification and crystallization. Pure flavonoid isolates were obtained with a purity level of 85%. The flavonoid isolate is in the form of a yellowish white powder and has an aroma. Qualitative identification confirmed that the isolate was positive for flavonoid compounds. The identification approach using the FT-IR spectrum indicates the presence of functional groups and chemical bonds in flavonoid compounds in the form of aldehydes, hydroxyls and ethers as markers for the presence of flavonoid compounds. Further studies are still being carried out to reveal the presence of single chemical compounds from the flavonoid group, to ensure pure chemical compounds as raw materials for medicine. The protocol for isolating flavonoids from frankincense resin is very necessary to obtain pure compounds as medicinal raw materials because they have antioxidant and antimicrobial properties.

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