



**Sudan University of Science and Technology**

**College Of Science**

**Department of Chemistry**



**Characterization of the Chemical Composition of the Ester from  
Sumatra Benzoin Gum**

**A thesis Submitted in partial fulfillment for the Requirement of the  
degree of Bachelor in Chemistry**

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## الآية

قَالَ تَعَالَى:

﴿يَرْفَعُ اللَّهُ الَّذِينَ ءَامَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۗ وَاللَّهُ بِمَا

تَعْمَلُونَ خَبِيرٌ ﴿١١﴾

صدق الله العظيم

المجادلة: ١١

## **Dedication**

This research work is dedicated to our respective parents who have been our constant source of inspiration. They have given us the drive and enthusiasm and without their love and support this project would not have been made possible.

To our families whom support us all the time.

## **Acknowledgement**

First of all we would like to thank **Allah** (God) who gave us strength, blessing, and courage during this study and during all of our life.

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Also we would like to thank all our colleagues, friends, and former lecturers at Department of Chemistry, Faculty of Science at SUST, Sudan.

## **Abstract**

In this study the chemicals composition of the ester from Sumatra benzoin resin which is naturally occurring resins was investigated. A qualitative test was utilized to differentiate between Siam and Sumatra resins. The resin was subjected to alkaline hydrolysis first which was followed by solvent extraction process. The yield of the separated ester was found to be equal to 14.35% (w/w). IR measurements confirmed that the main constituents of the esters are cinnamic acid and cinnamyl alcohol.

## ملخص البحث

هدفت هذه الدراسة لتحديد التركيب الكيميائي لمكونات الأستر المستخلص من راتنج السوماترا. أجرى اختبار كفي للتفريق بين راتنج السوماترا وراتنج السيام. و من ثم اخضع الراتنج الي تحليل في وسط قاعدي و الذي تلاه عملية استخلاص بالمذيب. استخدمت تقنية الأشعه تحت الحمراء ونقطة الانصهار للتعرف علي المكونات الكيميائيه للاسترز اوضحت النتائج ان كمية الاستر تساوي 14,35%(وزن/وزن). بينت نتائج الأشعه تحت الحمراء ان المكونات الأساسية للاستر هي حمض السنمك و الكحول السنمايل، كما اكد قياس نقطة الانصهار النتائج اعلاه.

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# Chapter One

## Introduction and literature review

### 1.1 Introduction

In recent years, increasing worldwide environmental awareness together with declining of petroleum resources have incited material scientists and engineers to look for alternative materials that are more sustainable, renewable, low cost, and environmentally friendly [1]. Natural resins are extracts of the vegetable or animal origin. These include rosin (gum, wood or tall oil rosins from tree and plants exudates; wood extracts; or by-products from paper manufacturing), fossil- resins such as amber; mined resins such as asphaltite; shellac assecration product from an insect; and their main derivatives. On the other hand, synthetic resins are defined as resulting sources from controlled chemical reactions such as polyaddition or polycondensation between well- defined reactants that do not themselves have the characteristics of resins. Synthetic resins are also obtained by polymerization of unsaturated monomers[2].

### Objective

The objective of this research work was to separate the ester from Sumatra benzoin resin and characterize the chemical composition of its constituents.

## **1.2 Resins**

They are natural or induced solid or semi- solid exudations from plants or from insects feeding on plants. They are characterized by being insoluble in water, mostly soluble in alcohol or ether, often uncrystallisable, and softening or melting at moderate heat forming sticky or adhesive fluid without volatilization or decomposition. They range in specific gravity from 0.9 to 1.25. Ignited in the air, they burn with a smoky flame, owing to the high carbon content in their molecule. They are usually the oxidized terpenes of the volatile oils of plants and owing to their insolubility in water, have little taste. Resins, when pure are usually transparent, when they contain water; they are opaque, and no longer hard and brittle. They are non- conductors of electricity, but when rubbed they become negatively electrified[3].

## **1.3 Distinguish between natural resins and prepared resins**

A clear distinction must be made between natural resins and prepared resins. A natural resin is one which occurs as an exudation e.g. mastic. A prepared resin may be made by extraction of the drug with alcohol, pouring the concentrated alcoholic percolate into an excess of acidified water, collecting, washing and drying the precipitate e.g., podophyllum and jalap resins. A prepared resin may also be derived from a natural oleoresin by driving off the volatile oil by steam distillation e.g., colophony. Careful distinction must be made between the above classes of resins and the so called synthetic resins. The latter are polymeric substances which are readily formed either by condensation or by addition of readily available common chemicals. For example, phenol and formaldehyde interact initially

to produce o- and p- hydroxyl benzyl alcohols which then condense to yield a large series of phenol- formaldehyde (Bakelite) resins.

(Bakelite) resins[3].

## **1.4 Resin combination**

### **1.4.1 Oleoresins**

Natural oleoresins are mixtures of volatile oils and resins and therefore they are liquids or semi liquids substances depending on the amount of the volatile present. Turpentine, copaiba and Canada balsam are examples of this group [3].

### **1.4.2 Gum Resins**

These are natural mixtures of gum and resin, usually obtained as exudations from plants, as myrrh[3].

### **1.4.3 Oleo-gum- resin**

Resin may occur in combination with volatile oil and gum for example asafetida[3].

### **1.4.4 Glycoresins**

Resin may be combined in a glucosidal way with sugars as the resin of the convolvulaceae being called Glycoresins which are found in Ipomoea, Jalap and podophyllum[2].

### 1.4.5 Balsams

They are resinous substances that contain the aromatic balsamic acids i.e. benzoic acid or cinnamic acid or both or esters of these acids, Balsams usually contain small amount of volatile oil [3].

#### 1.4.5.1 Balsam of Tolu (*Balsamum toluatanum*)

Balsam of Tolu is balsam obtained by making incisions in trunk of *Myroxylon balsamum*, Family Leguminosae [3].

#### 1.4.5.2 Balsam of Peru (*Balsamum peruvianum*)

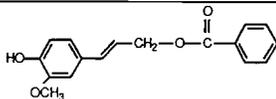
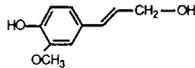
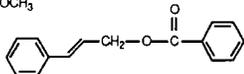
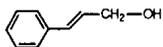
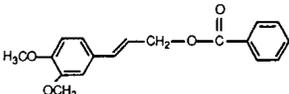
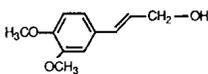
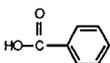
Balsam of Peru is a balsam exuded from the trunk of *Myroxylon pereirae*, family Leguminosae, after the trunk has been beaten and scorched [3].

#### 1.4.5.3 Benzoin

Benzoe tonkinensis is a natural complex balsamic resin obtained from a native tree in Laos, *Styrax tonkinensis* (Pierre). It is collected directly from the tree, clean and sorted into four grades according to size. All grades have similar chemical composition [4,5,6]. There are two types of benzoe tonkinensis, *Styrax tonkinensis* and Sumatra benzoin. In *Styrax tonkinensis* there are Siam benzoin and Lao benzoin refer to the same product from *Styrax tonkinensis* and the former name is used almost universally outside Lao PDR when it is necessary to distinguish benzoin of Lao origin from that produced in Indonesia (Sumatra benzoin). The name originates from the previous importance of Thailand (old name Siam) as an international exit point for benzoin. The latter name is used for the benzoin specifically

produced in Lao PDR, the major producer of Siam or Lao benzoin, a balsamic resin obtained from the yan tree (*Styrax tonkinensis*), which is native to Southeast Asia. While Sumatra benzoin, is derived from two other *Styrax* species: *S. benzoin* and *S. paralleloneurum*, both also Southeast Asian trees. Indonesia, specifically north Sumatra, is the only producer of Sumatra benzoin. The scale of production of the two types of benzoin is very different[7]. Benzoin comes from tree species of the genus *Styrax* in the family *Styracaceae*. *Styrax* contains about 130 species of trees and shrubs occurring in tropical to temperate climates. Three centres of distribution are described: southeastern Asia, southeastern North America to South America, and a single species in the Mediterranean. Siam benzoin is obtained from *S. tonkinensis* (Pierre) Craib ex Hartwiss. Sumatra benzoin is collected from two species: *S. benzoin* Dryand. And *S. paralleloneurum*/Perkins (sometimes spelled paralleloneurus). The structures of the main components are shown below (Table 1).

**Table 1: Shows the structures of the main components of benzoin.**

Compound	Structure
Coniferyl benzoate	
Coniferyl alcohol	
Cinnamyl benzoate	
Cinnamyl alcohol	
3,4-dimethoxycinnamyl benzoate	
3,4-dimethoxycinnamyl alcohol	
Benzoic acid	

### 1.5 Description of Sumatra trees

The flowering of the trees in a district is gregarious and evidently takes place only after marked dry weather, often at the beginning of the year. The flowering only lasts for a few days during which the trees give off a fragrant like Lily of the Valley. They have blue fruits that are eaten by birds and thus distributed by them. The blue color of the fruit is caused, not by a pigment but by the structure of the cuticle which reflects blue light [8].



**Figure 1: Shows benzoin tree and its resin**

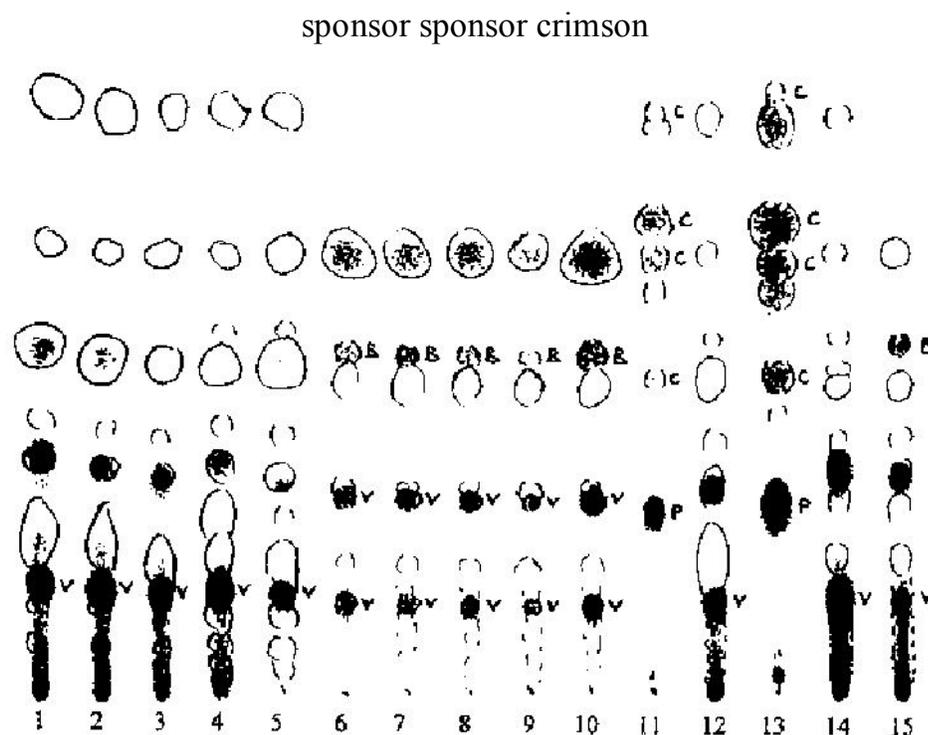
### 1.6 Studies used to distinguish between Siam and Sumatra benzoin

(i) When about 0.5 gm of powdered benzoin is warmed with 10ml of potassium permanganate solution, a faint odor of benzaldehyde is developed only with Sumatra benzoin but not with the Siam benzoin.

(ii) Digest about 0.2gm of the coarsely powdered benzoin with 5ml of ether for about 5 minutes decant about 1ml of the ethereal solution into a porcelain dish containing 2-3 drops of sulphuric acid and mix carefully, a deep purplish-red color with Siam benzoin and deep reddish brown color in produced with Sumatra benzoin.

### (iii) Thin layer chromatography

Fifteen samples of benzoin collected during the regional fieldwork were analyzed using TLC. TLC can be used qualitatively to identify the same compounds in different samples. If standards are available then it may be possible to identify the compounds themselves. Some spots fluoresce under ultra-violet light. If a chemical spray reagent is used to detect the spots, different colors may be produced by different compounds, and this, too, assists identification. The intensity (size) of the spot is proportional to the concentration of the compound in the mixture and so quantitative analysis is also possible under carefully controlled conditions. The results are shown below:-



**Figure 2: show separation of components of benzoin resin by TLC**

Where B = blue, V = violet, P = pink, C = crimson

So according to this study it was found that the Sample identification:

- 1 Sumatra, grade 1, ex Singapore [company A]
- 2 Sumatra, grade 2, ex Singapore [company A]
- 3 Sumatra, grade 3 (dust), ex Singapore [company A]
- 4 Sumatra, grade 1, ex Singapore [company B]
- 5 Sumatra, grade B, ex Indonesia [company C]
- 6 Siam, grade A, ex Lao PDR [company D]
- 7 Siam, grade B, ex Lao PDR [company D]
- 8 Siam, grade C, ex Lao PDR [company D]
- 9 Siam, grade D (dust), ex Lao PDR [company D]
- 10 Siam, mixed grade, freshly collected, ex Lao PDR
- 11 Sumatra block (low quality), ex Kuala Lumpur
- 12 Sumatra block (good quality brand), ex Singapore [company A]
- 13 Sumatra block (low quality brand), ex Singapore [company A]
- 14 Sumatra, mixed (low quality) ex Singapore [company B]
- 15 Siam, ex Bangkok

Grade 1, very largetears, yellow-orange, without any foreign particles; Grade 2, large tears, orangeyellow; Grade 3, small tears, orange; Grade 5, very small and agglomerated tears, almost powder-like, deep red, contains a lot of foreign particles, such as pieces of wood and bark [1].

It was shown that silica gel 60 F254 HPTLC (high performance TLC) plates give an improved separation of spots compared to the TLC plates used here, also furthermore, use of a methanolic dip solution containing the anisaldehyde detection reagent (into which the HPTLC plate is dipped for a few seconds) was found to give much better results than applying the reagent as a spray: a more uniform application of the reagent to the plate was achieved, resulting in more sharply defined spots. It is preferable, also, to substitute dichloromethane for benzene in the solvent system [9].

### **1.7 Chemical composition of Siam and Sumatra benzoin**

The chemical compositions of the two types of benzoin account for their sensory characteristics and determine the uses to which they are put. There are both similarities and differences in composition and this means that although they are both used for flavour and fragrance purposes, they often go into different parts of the markets. Both contain mixtures of organic acids and esters, along with numerous other -mostly minor -components, and both can be described as balsamic in odor [7]. However, in Siam resin the chief constituents are benzoic acid (15-45 %) and coniferyl benzoate (15-60%), with lesser amounts of vanillin (<5%), benzyl benzoate (<2%), 2-hydroxy-1-phenylethanone and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone [4, 5, 10]. Benzoic acid, vanillin and benzyl benzoate are identified and benzoic acid is quantified by gas chromatography. The second type of commercial benzoin, Sumatra benzoin, is derived from two other *Styrax* species: *S. benzoin* and *S. paralleloneurum*, both also Southeast Asian trees. Indonesia, specifically north Sumatra, is the only producer of Sumatra benzoin. Its main components are cinnamic acid and its esters (such as coniferyl cinnamate and cinnamyl cinnamate). Vanillin is present in both types of benzoin resins and gives rise to its familiar vanilla odour (most readily detected in the Siam type) [5, 10].

### **1.8 Benzoe Sumatra**

Benzoe Sumatra occurs in masses consisting of opaque creamy white tears embedded in a dull grayish-brown or sometimes reddish-brown matrix. It is hard and brittle and the fractured surface is dull and uneven. It possesses an agreeable balsamic odor and slightly acrid taste, when gradually heated it melts and evolves whitish irritating fumes of benzoic and cinnamic acids, when a little of the crushed resin is warmed with dilute sulphuric acid and potassium permanganate, Benzaldehyde is evolved indicating the presence of cinnamic acid in the drug [3].

Benzoe Sumatras obtained by solvent extraction of resin from styrax benzoin dryand,a tree growing predominantly on island of Sumatra. It is dark brown, viscous liquid with a warm, powdery, sweet-balsamic odor. It is main volatile, odor-determining components are derivatives of benzoic, cinnamic and vanillin[11].

Benzoe Sumatra resinoid is used in perfumery, mainly as a fixative with a warm, balsamic note, also Sumatra benzoin (and, to a lesser extent, Siam benzoin) is used quite widely inpharmaceutical preparations as an ingredient of inhalations for the treatment of catarrh andin topical preparations for its antiseptic and protective properties. Benzoin is also used intraditional Chinese medicine. In addition to that Sumatra as well as Siam isused to be included in the British Pharmacopoeia (BP, 1980), for whicha minimum content of 25% total balsamic acids was required (calculated as benzoic acid forSiam benzoin and cinnamic acid for Sumatra benzoin). The amount of (90%) ethanolinsoluble matter allowable was less for Siam benzoin (not more than 5%) than Sumatrabenzoin (not more than 20%). The most recent British Pharmacopoeia (BP, 1993) onlydescribes Sumatra benzoin; included in the specification is a test for checking the absence ofdamar.An FAO specification for "Benzoin gum" describes requirements of both types of benzoin foruse as a flavoring agent[10].

## **1.9 Cinnamic acid and its esters**

Cinnamic acid derivatives are important compounds with a wide range of biological activities which includesantibacterial,antifungal, antioxidant, anti-inflammatory,and antitumor.Some cinnamic acid derivatives arenaturally occurring substances found in various plants.Cinnamic acid can also be found in free form, but itis especially common in the form of esters. Cinnamicesters are obtained from various plant sources and arevery

important in perfumery, the cosmetic industry, and pharmaceuticals. Methyl caffeate is found in *Gaillardia pulchella*, *Gochnatia rusbyana*, *Notopterygium incisum*, and the fruits of *Linum usitatissimum* and is reported to possess both antitumor and antimicrobial activities. Ethyl 3, 4, 5-trimethoxycinnamate, found in *Piper longum*, has an important role in controlling inflammatory diseases [12].

## Chapter Two

### Experimental

#### 2.1 Materials

The sample (*Sumatra resin*) was purchased from the local market (Alsoug al-arabi-Khartoum, Sudan) and it was ground to a fine powder and used without further treatments.

Diethyl ether ( $\text{CH}_3\text{CH}_2\text{OCH}_2\text{CH}_3$ , Assay = (GLC)>99%). Ethyl Acetate ( $\text{CH}_3\text{COOC}_2\text{H}_5$ ,  $M_{\text{wt}} = 88.11$ , Assay (GC) min =99%, density = 0.899 — 0.902g/cm<sup>3</sup> by ALPHA chemikA). Ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ,  $M_{\text{wt}} = 46$  g/mol, Assay=95%, density at 25<sup>0</sup>c=0.789g/cm<sup>3</sup>, LOBLchemie). n-Hexane ( $\text{C}_6\text{H}_{14}$ ,  $M_{\text{wt}} = 86.18$  g/mol, Assay not less than 85%, density at 20<sup>0</sup>C =0.655—0.665g/cm<sup>3</sup>, ALPHA ChemiKA). Hydrochloric acid (HCl,  $M_{\text{wt}} = 36.46$  g/mol, Assay (acidimetric)35—38%, density at 20<sup>0</sup>C about 1.18g/cm<sup>3</sup>, LOBA Chemie). Methanol ( $\text{CH}_3\text{OH}$ , (G.C.) 99.5%wt.per ml at20<sup>0</sup>C =0.790-0.793g/cm<sup>3</sup>, LOBLchemie). Potassium Hydroxide ( $M_{\text{wt}}=56.11$ , assay=85%, laboratory Reagent).

#### 2.2 Methods

##### 2.2.1 Differentiation between Siam and Sumatra resins

(i) 0.5 gm of powdered benzoin was warmed with 10ml of potassium permanganate solution. Faint odor of benzaldehyde was developed.

(ii) 0.2gm of coarsely powdered benzoin was digested with 5ml of ether for about 5 minutes. 1ml of the ethereal solution was decanted into a porcelain dish containing 2-3 drops of sulphuric acid and mixed carefully. Development of a deep reddish brown color was observed.

## **2.2.2 Alkaline hydrolysis of benzoin gum**

2.0508g of the powdered sample were weighed and transferred to a round bottom flask. A mixture of 10% alcoholic potassium hydroxide and 10% aqueous potassium hydroxide were added to the sample in the ratio 2:3, respectively, and refluxed for four hours at 60-80<sup>0</sup>C. The solution was cooled to room temperature and the solid residue was separated by decantation. Then it was extracted three times using n-hexane and the organic layers were collected. The remaining aqueous solution was acidified by HCl till the pH was around 2 and extracted three times with n-hexane and the organic layers were also collected. The organic solvent was left to evaporate at room temperature and the dried solid material was weighed. The percentage of the ester was calculated using the following equation:

$$\text{Percentage of the ester (\%)} = (\text{wt. of ester/wt. of original sample}) \times 100$$

## **2.3 Characterization methods**

### **2.3.1 IR measurements**

FTIR transmittance spectra of the separated components were obtained using a Shimadzu FT-IR spectrometer in the wavenumber range of 4000 to 500 cm<sup>-1</sup>. The powdered sample was thoroughly mixed with potassium bromide, pressed to make a pellet, and then scanned to obtain its IR spectrum.

### **2.3.2 Melting point method**

Small amount of the sample was placed in the bottom of a narrow capillary tube which had been closed at one end then the melting point was determined by using melting point apparatus.

## Chapter Three

### Results and discussion

#### 3.1 Differentiation test

When the resin was treated with potassium permanganate solution a faint odor of benzaldehyde was developed. Furthermore, the treatment of the ethereal extract of the sample with sulphuric acid gave a deep reddish brown color which further confirms that the sample is Sumatra resin.

#### 3.2 The percentage of the ester in Sumatra resin

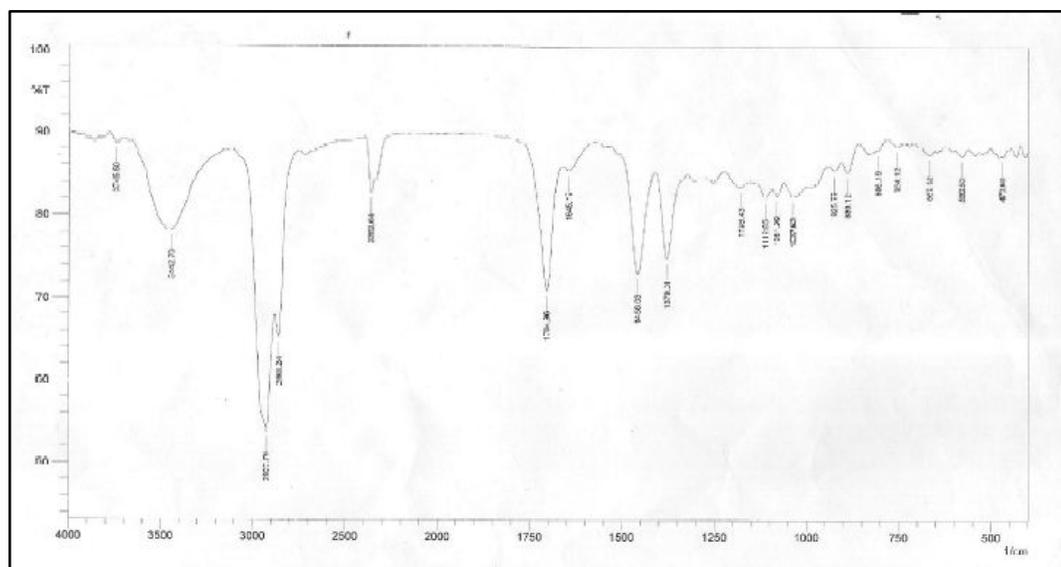
**Table (2):** Represents the weights of the sample and the separated components as well as the percentage of the ester.

The Weight of the sample	The weight of the acid	The weight of the alcohol	The percentage yield of the ester
2.050g	0.1302g	0.1642g	14.355%

#### 3.3 IR measurement

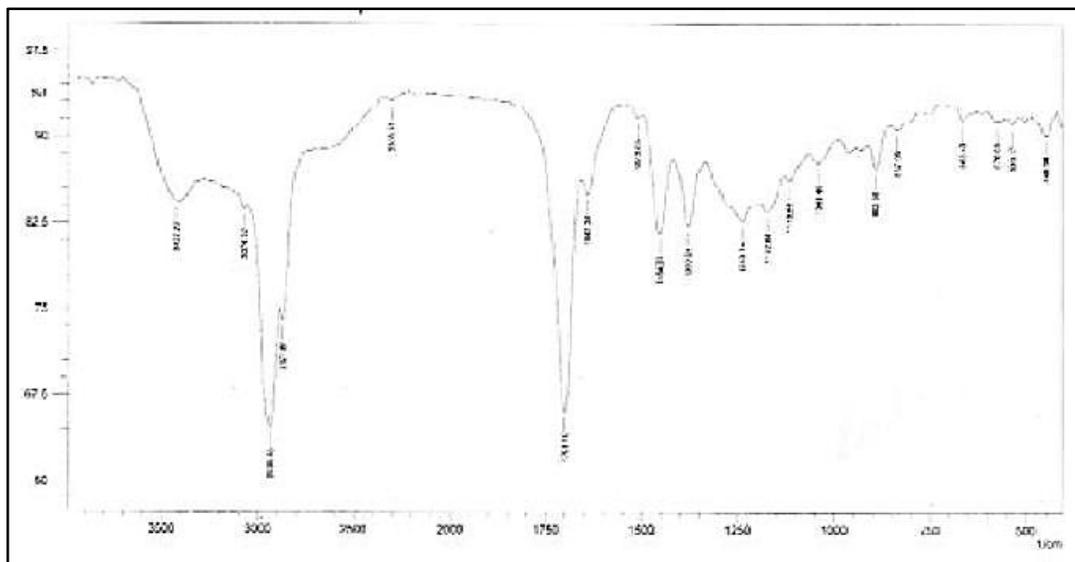
FTIR analyses were carried out to confirm the presence of the functional groups of cinnamic acid and cinnamyl alcohol which were reported to be the main constituents of the ester [5, 11]. Figure 4 represents the FTIR spectrum of cinnamic acid. The main characteristics functional groups of cinnamic acid are: carbonyl group, C=C aromatic, C=C alkenic, C-H aromatic, CH alkenic, OH group, and, C-O. As can be seen from the spectrum, the broad and intense peak in range between 3200 and 3600  $\text{cm}^{-1}$  is due to the absorption of -OH group. strong sharp peak at 1701  $\text{cm}^{-1}$  due to

absorption of (C=O), weak peak at  $1645\text{cm}^{-1}$  due to absorption of (C= alkene), weak peak at  $1512.09$  and  $1454.20\text{cm}^{-1}$  due to (C=C aromatic),  $1240\text{cm}^{-1}$ ,  $1041.49\text{cm}^{-1}$ ,  $837.05$  and  $665.40\text{cm}^{-1}$ . It is obvious that these results agree with the results obtained from the standard cinnamic acid from literature.[13]. While the main characteristic functional groups of cinnamyl alcohol are: carbonyl group, C=C aromatic, C=C alkenic, C-H aromatic, CH alkenic, OH group, and, C-O. As can be seen from the spectrum, weak peak at  $1645.17\text{cm}^{-1}$  due to absorption of (C= alkene), weak and sharp peak at  $1379.01\text{cm}^{-1}$  and  $1458.08\text{cm}^{-1}$  due to absorption of (C= c aromatic), weak and broad peak at  $3442\text{cm}^{-1}$  due to the absorption of (OH str. alcohol).



**Figure 3: show the IR spectrum of the cinnamyl alcohol**

Those results obtained from IR confirmed that the acid is cinnamic acid and the alcohol is cinnamyl alcohol.



**Figure 4: show the IR spectrum of cinnamic acid**

### **3.4 Melting point**

The melting point of cinnamic acid was found to be equal to 148<sup>0</sup>C which is almost typical to the standard value reported for cinnamic acid in the literature.

## **Conclusion**

The present study deals with characterization of the chemical composition of the ester from Sumatra benzoin gum. The results demonstrated that the main components are cinnamic acid and cinnamyl alcohol with the percentage of ester equals 14%.

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