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Gambir Quality from West Sumatra Indonesia Processed With Traditional Extraction

Tuty Angraini, Neswati and Alfi Asben

Faculty of Agricultural Technology Andalas University, Kampus Limau Manis 25163
Corresponding author : tuty@ae.unand.ac.id

Abstract . Gambir is dried extract of leaves and branches of *Uncaria gambir*. Pesisir Selatan and Lima Puluh Kota were the central place in produce gambir in West Sumatra, Indonesia. This study identified gambir from both Pesisir Selatan and Lima Puluh Kota West Sumatra Indonesia for its quality. The analysis of gambir were the antioxidant activity, total polyphenol content and catechin content. Catechin content determined with High Performance Liquid Chromatography (HPLC). The result showed that the antioxidant activity, total polyphenols and the average of catechin content gambir from Pesisir Selatan and Lima Puluh Kota Region were 91.33 % and 91.55 %, 197.35 mg/m GAE and 182.00 mg/g GAE and 50.87 % and 55.40% respectively. The conclusion were the antioxidant activity, total polyphenols and catechin content of gambir from Pesisir Selatan and Lima Puluh Kota are influenced by the raw materials.

1. Introduction

Catechin and its derivate have positive function for human health due to their antioxidant activity and have wide ranged purposes for food preservative, cosmetic and medicine [1,2,3,4]. For food preservatives, catechin act as antimicrobial, for cosmetics, catechin believed to have ability as anti-aging, for medicine , catechin effective against cancer, tumor, atherosclerotic and other diseases caused by oxidative stress [5,6,7,8]. Catechin could also be used as traditionally medicine, especially in West Sumatra, such as sore throat, diarrhea and stomach. One of the plant that potential to produce catechin and its derivate is *Uncaria gambir* plant. *Uncaria gambir* is a native plant from West Sumatra. The dried extract from leaves and branches of *Uncaria gambir*, known as gambir. Gambir have great potential as formula to make rigid foams, as green inhibitor of calcium carbonate and to produce biosorbents [9,10,11].

In West Sumatra, Pesisir Selatan and Lima Puluh Kota were the central region to produced gambir. *Uncaria gambir*, as a native plant is a source of catechins. Gambir produced with traditional extraction, named with mangampo is a whole process to get gambir. In Pesisir Selatan and Lima Puluh Kota region the steps to get gambir were: harvesting, steaming, pressing, clumping, removing water, forming and drying. These processing was the step or procedure of ‘mangampo’. Mangampo is unique traditional process to produced gambir [12]. These extraction process influenced the quality of gambir, for example the raw material and the mechanical energy to press the extract. The aimed of this study was to identification of gambir produced in Pesisir Selatan and Lima Puluh Kota region. The analysis of antioxidant activity, the total polyphenol content and the catechin content were determined as the gambir quality of each region.



2. Materials and Methods

2.1. Materials

Raw material : Gambir purchased from farmer directly in Pesisir Selatan and Lima Puluh Kota Region. Chemicals : 1-Diphenyl-2-picrylhydrazyl (DPPH), Ethanol, Methanol, Folin ciocalteu, Na₂CO₃, Gallic acid, distilled water, acetonitrile, acetic acid and ethyl acetate.

2.2. Methodology

The research design used in this study is exploratory method. Data are calculated as standard deviation (sd).

2.3. Analysis

2.3.1. Antioxidant Activity [13]:

A total of 1 g of sample is put into a test tube containing 10 ml of methanol. The solution was homogeneous in ultrasonic for 30 minutes. Samples were prepared at concentrations of 1 mg / ml, 0.1 mg / ml and 0.01 mg / ml. From each sample concentration, as much as 1 ml was put into a test tube and 20 ml of methanol and 1 ml of DPPH were added. Blanks are prepared by mixing 3 ml of methanol and 1 ml of DPPH. The mixture was kept at an ambient temperature for various incubation times 30 minutes prior to the measurement of the absorbance at 517 nm (A_{517 nm}). All measurements were performed in triplicate. The formula as follow :

$$\text{Antioxidant Activity} = \left[\frac{(\text{control absorbance} - \text{extract absorbance})}{\text{control absorbance}} \right] \times 100\%$$

2.3.2. Total Polyphenol [14] :

A sample of 1 g was weighed into a test tube containing 10 ml of methanol. The solution was homogenized using ultrasonic for 30 minutes. Samples were prepared at concentrations of 1 mg / ml, 0.1 mg / ml and 0.01 mg / ml. Each sample at each concentration was taken as much as 1 l and added 2 ml of distilled water, 1 ml of folin-ciocalteu reagent and homogenized. Next Na₂CO₃ was added. Samples were allowed to stand for 1 hour, then measured by spectrophotometer at a wavelength of 723 nm. Standard curves were made with 15 ppm, 20 ppm, 25 ppm, 30 ppm, 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, 3000 ppm, 3500 ppm and 4000 ppm. The total content of polyphenols is expressed in mg GAE / g.

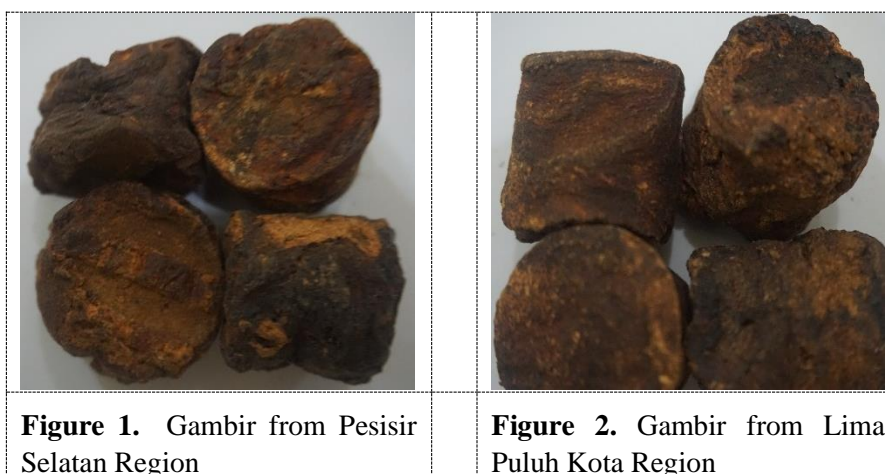
2.3.3. Catechin Content

Sample preparation of gambir : 1 g of gambir added with 100 ml mixed of ethyl acetate and ethanol (50% ; 50%). Then heat the solution at 60°C for 6 hours, and filtered. Evaporated the gambir solution at 45°C. Add 20 ml methanol to dried samples, then add 1 ml with 10 ml methanol. A 20 µl was subjected into HPLC analysis using C₈-DAD column, at wave length 280 nm, column temperature 40°C and flow rate 0.5 ml/min. The elution was performed using acetonitrile : TFA 0.1% (in acetic acid 0.1%) (1 : 1 / v/v).

3. Result and Discussions

Gambir produced from Pesisir Selatan and Lima Puluh Kota region, have the similar method to produced gambir. The process to produce gambir from harvesting to drying process use the same methods. But actually, there were difference due to their appearance, and this difference caused by the various harvesting time, different treatments on *Uncaria gambir* plant (maintained or not) and the water quality that used for steaming. It can conclude that the Region did not affected the quality, but the raw material and water quality influenced gambir quality [12].

Figure 1 and Figure 2 showed gambir from Pesisir Selatan and Lima Puluh Kota. The sample were selected randomly from both region.



As seen on Table 1 and 2, there were no significance different in appearance between gambir from Pesisir Selatan and Lima Puluh Kota region. Gambir have dark brown to black color in the surface, but soft brown inside. The colour of the gambir have different color, influenced by the raw materials and water to steam the leaves and branches. Usually gambir dried with sun drying, but if the weather are cloudy or rainy, gambir will put in the processing room. The steaming process used wood as burner which produced smoke. These some will influence gambir colour, it will turned into black on the surface.

3.1. Antioxidant Activity

Tabel 1 showed the antioxidant activity content of gambir from Pesisir Selatan and Lima Puluh Kota region.

Table 1. The Antioxidant Activity of Gambir

	Concentration		
	1mg/ml	0.1 mg/ml	0.01 mg/ml
Pesisir Selatan (%)	91.33±1,03	36.69±0.97	3.87±1.10
Lima Puluh Kota (%)	91.55±0.46	39.43±0.65	9.33±0.53

Table 1 showed that the antioxidant activity for both Region were similar due to the same processing to produced gambir. According to Anggraini et al [12] the processing to produced gambir from Pesisir Selatan and Lima Puluh Kota, West Sumatra was similar, and the traditional processing called 'mangampo'. The antioxidant activity of gambir influenced by the concentration, higher concentration resulted high antioxidant activity. Catechin as one of polyphenol was the major compounds presence in gambir, have the ability as antioxidant¹. The same result showed that gambir as natural antioxidant as source of phenolic and flavonoid, which potential to reduce aging and as therapeutic agent¹⁵. One of the factor influence the antioxidant compound in gambir was the raw material. The raw material to produced gambir is leaves and branches of *Uncaria gambir* Roxb. Some of the *Uncaria gambir* plantation were protected from shrub or competitor plant, but at another plantation *uncaria gambir* growth in not appropriate condition, where many competitor caused less fertilizer. These situation influence the synthesise of functional compounds.

3.2. Total Polyphenols

Total polyphenols gambir from Pesisir Selatan and Lima Puluh Kota Region showed by Table 2.

Table 2. The Total Polyphenols of Gambir

	Concentration		
	1mg/ml	0.1 mg/ml	0.01 mg/ml
Pesisir Selatan (mgGAE/g)	197.35±9.21	44.18±2.72	25.93±2.83
Lima Puluh Kota (mgGAE/g)	182.00±6.35	34.49±1.65	17.15±2.71

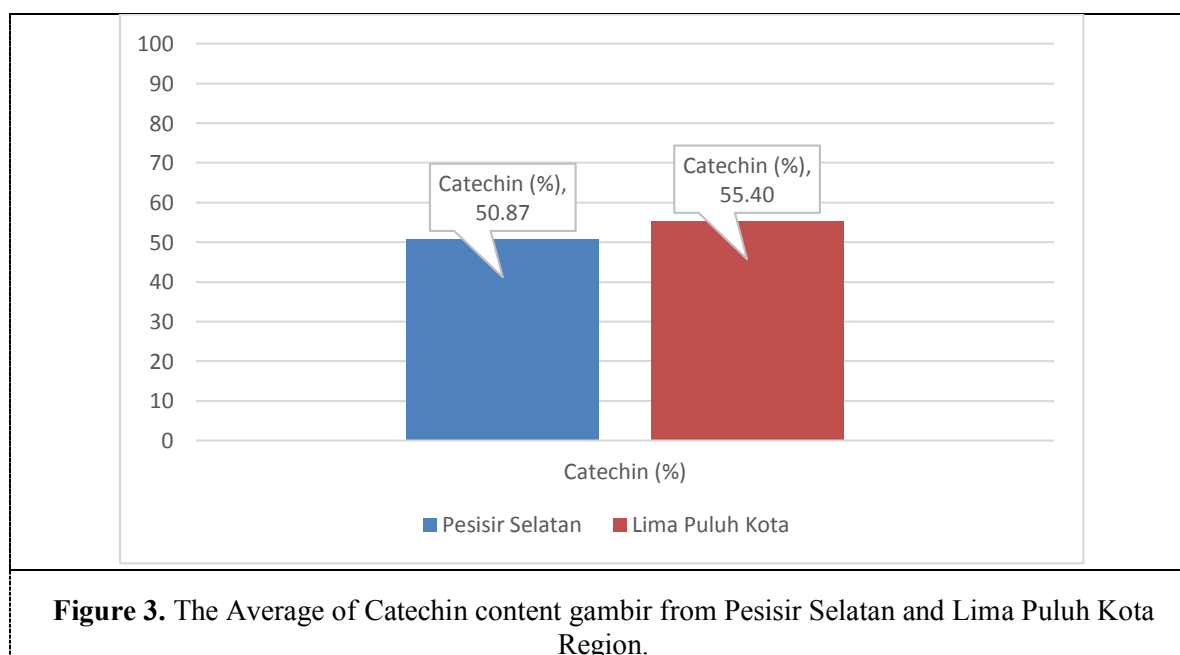
±sd

Polyphenols in Pesisir Selatan and Lima Puluh Kota region were 19.35 mgGAE/g and 182.00 mgGAE/g respectively. Polyphenols are bioactive compounds that responsible for the antioxidant activity in gambir. Same result as the antioxidant activity, the polyphenols in gambir increased by increasing of the concentration. Another research reported that total phenolic content in gambir was 18.37 mg GAE/g and flavonoid content was 5.82 mg RE/g¹⁵. Polyphenols compounds presence in gambir were catechin, epicatechin, tannin [1,16].

High amount of polyphenols in gambir resulted in high quality of gambir. High gambir quality will increase the price of gambir. The extraction of gambir from West Sumatra use hot extraction to produced gambir. High temperature break the cell walls and catechin separate by mechanical extraction. The use of hot extraction recommend to have high yield in plant [17].

3.3. Catechin Content

Catechin content in gambir from Pesisir Selatan and Lima Puluh Kota region showed in Figure 3.



The best method to extract gambir is using etylacetate, with temperature 70°C, resulted 97.40% catechin. The same result showed by another research , where etyl acetate is the best solvent to extract gambir^{18,19}. A research conducted by Zebua et al [16] the extraction of gambir with some solvents resulted 1.85% catechin with water, 1.98% catechin with acetate and 1.93% catechin with ethanol¹⁶.

To measured catechin, researcher used HPLC method. The chromatogram of gambir shown in Fig 4 until 9. The HPLC measurement used two methods to determined the optimal measurement for catechin content in gambir.

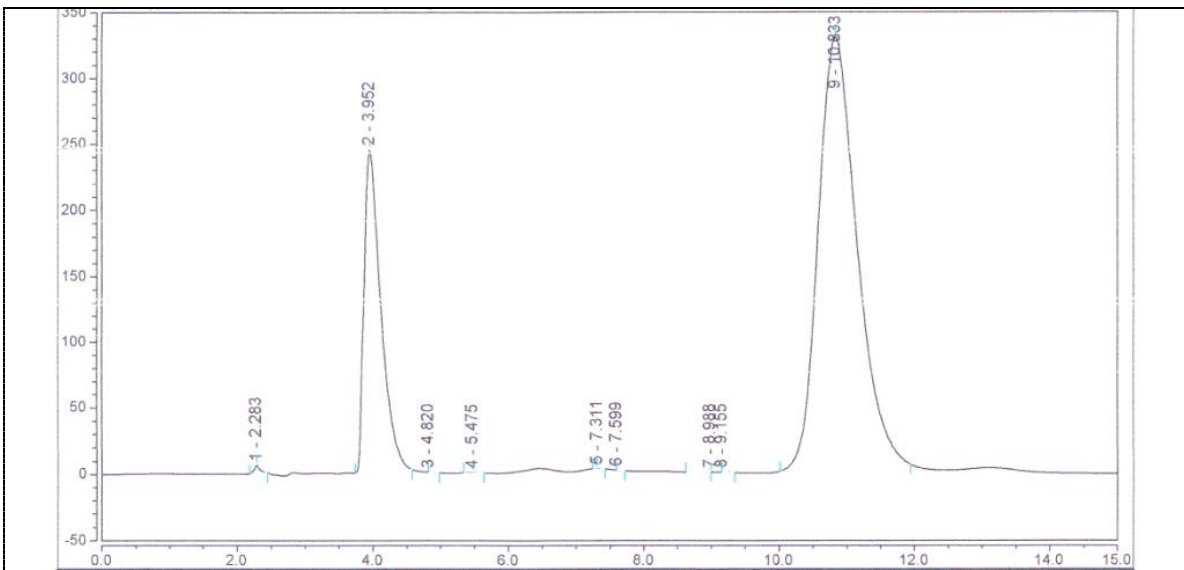


Figure 4. Chromatogram of catechin from Pesisir Selatan Region (Siguntur Tuo)

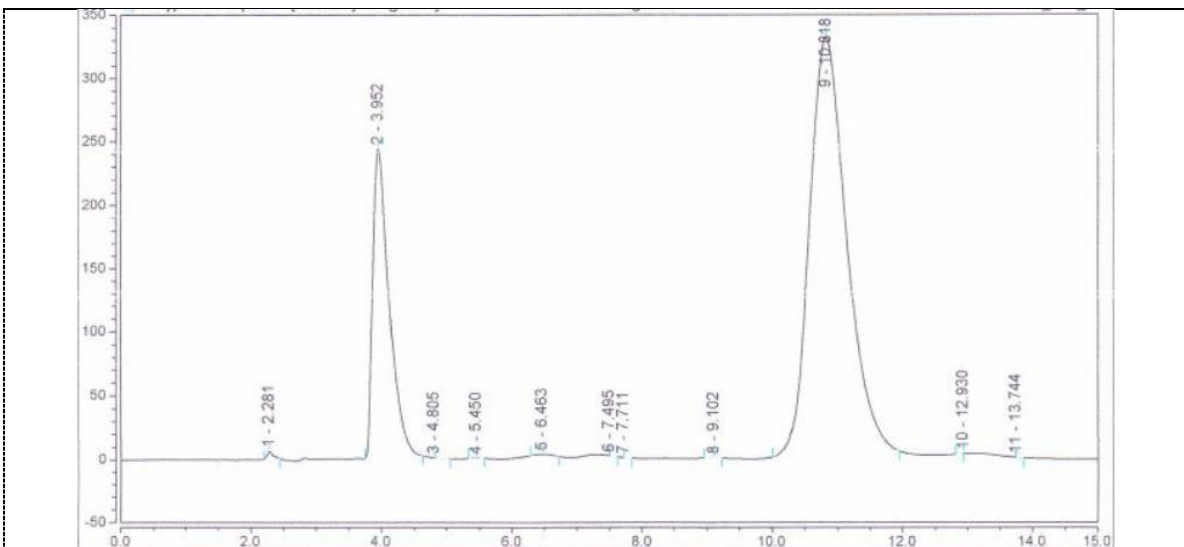


Figure 5. Chromatogram of catechin from Pesisir Selatan Region (Siguntur Mudo)

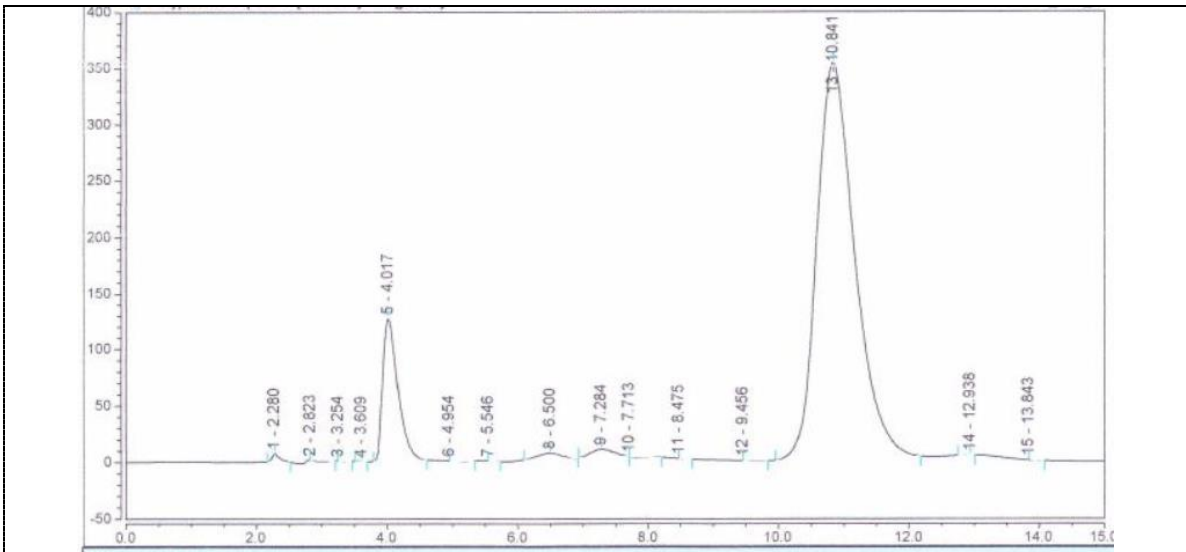


Figure 6. Chromatogram of catechin from Pesisir Selatan Region (Barung-Barung Belantai)

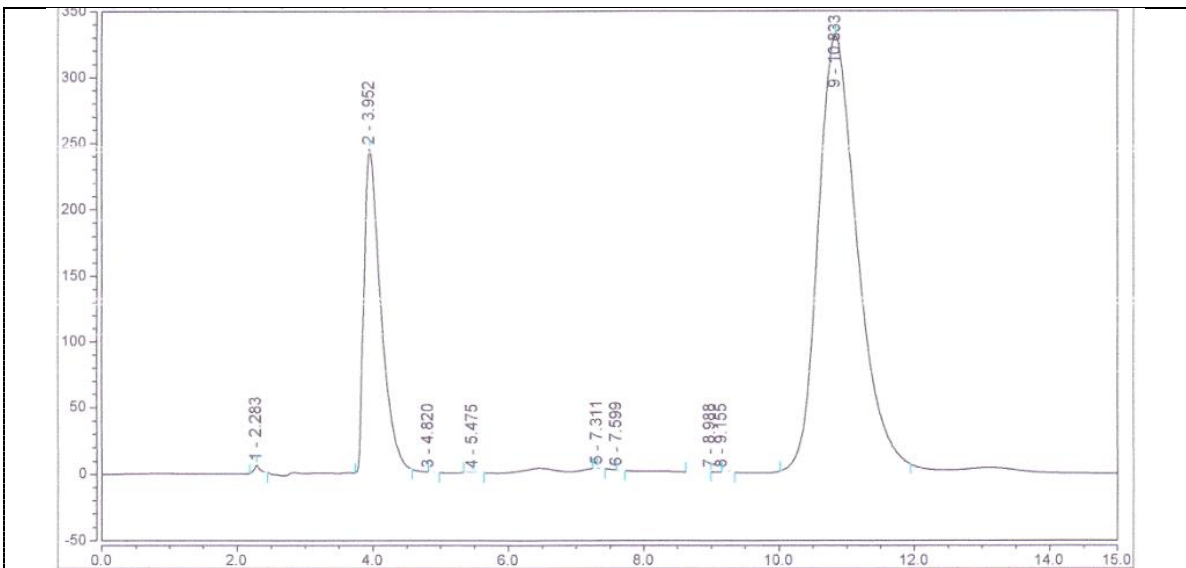


Figure 7. Chromatogram of catechin from Lima Puluh Kota Region (Jorong Bio-Bio)

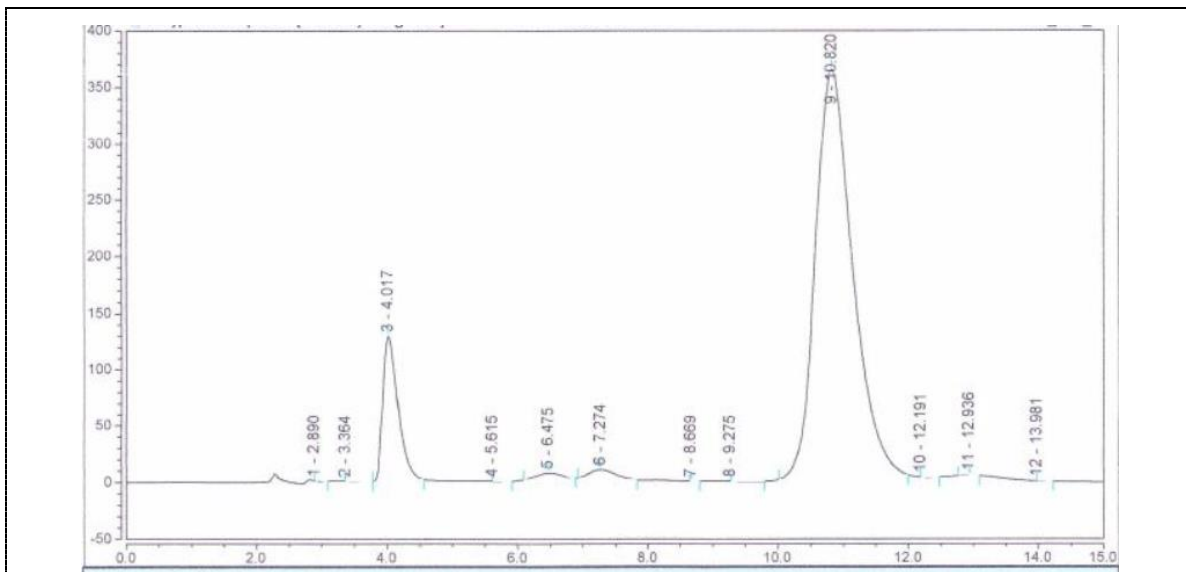


Figure 8. Chromatogram of catechin from Lima Puluh Kota Region (Simpang Kapuak)

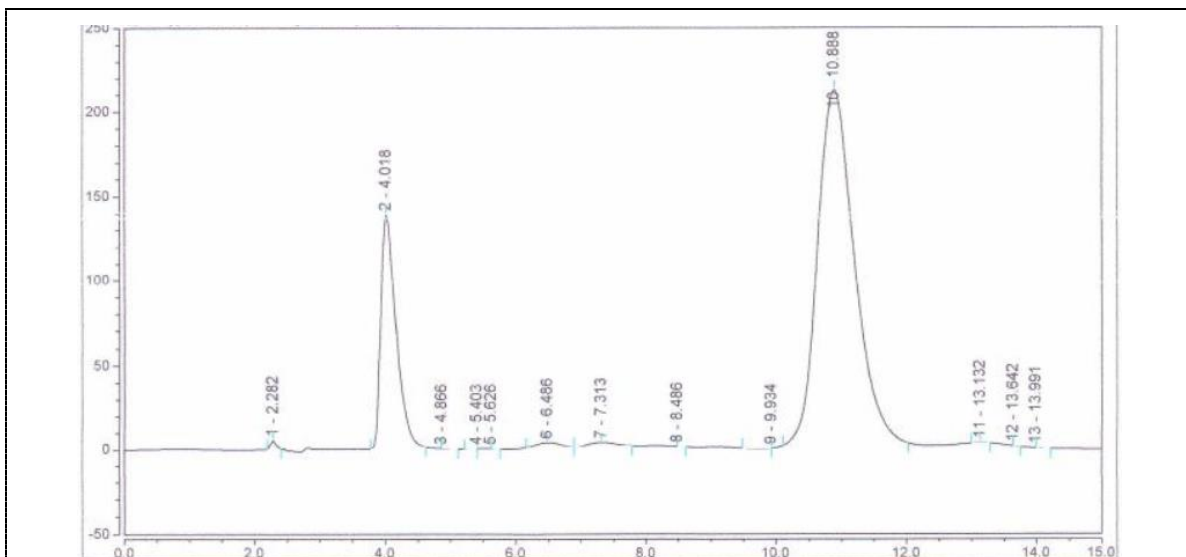


Figure 9. Chromatogram of catechin from Lima Puluh Kota Region (Muaro Paiti)

Table 3. Epicatechin and Epigallo catechin of gambir

	Epicatechin (ppm)	Epigallo catechin (ppm)
Pesisir Selatan Region		
Siguntur Mudo	235.9	9.3
Siguntur Tuo	845.4	10.7
Barung-barung Belantai		
Lima Puluh Kota	372.7	13.7
Jorong Bio-bio	550.7	23.5
Simpang Kapuak	442.3	6.1
Muaro paiti	447.5	9.6

HPLC is the best methods to analyse the catechin content compared with volumetric methods. There were many mobile phase that researcher used to measured catechin in gambir. Catechin as the major compounds in gambir have many function in food industry or other industry, such as pharmacology. Because the wide range purpose of gambir, this research will very important for another research to develop gambir extraction, especially which is produced from local farmers. Furthermore, researcher will determine catechin derivate in gambir.

4. Conclusion

The antioxidant activity, total polyphenols and catechin content of gambir from Pesisir Selatan and Lima Puluh Kota Region were 91.33 and 91.55 %, 197.35 and 182.00 mg/g GAE and 50.87 and 55.40% respectively. Gambir quality from Pesisir Selatan and Lima Puluh kota influenced by the raw materials.

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