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Characterization of Simplicia and Ethanol Extracts of Bark of Asam Kandis (*Garcinia cowa* Roxb)

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Abstract

Characterization of simplicia and ethanol extract from bark of Garcinia cowa Roxb has been done on plants taken from three different regions: Solok, Padang, and Padang Pariaman. Characteristics are tested according to the procedures listed in Pharmacopoeia Herbal Indonesia. The purpose of this study was to look at the characteristics of each sample taken from different regions. The results showed that the simplicia of Garcinia cowa Roxb stem bark was a rather coarse powder, dark brown, distinctive smell, and biting taste. The simplicia microscopic test shows that there are fragments of simplicia, i.e. calcium oxalate crystals of rosette form, cork tissue, sclerenchyma fibres. The physicochemical parameter of simplicia shows that drying drift is not more than 10%, total ash content not more than 6.66%, acid soluble ash content not more than 0.21%, water soluble extract not less than 11.71%, soluble extract in ethanol not less than 16.5%. Chemical content test showed that chromatogram pattern with chloroform-ethyl acetate eluate (9: 1) yielded Rf values of each region were 0.39, 0.39, and 0.38. The total phenolic content in the simplicia of each region was 11.44%, 9.75%, and 9.78%. The α-mangostin levels in the simplicia of each region were 0.06%, 0.05%, and 0.04%. The ethanol extract from Garcinia cowa Roxb stem bark is a thick, dark red extract, distinctive smell and bitter taste. The yield of the extract is not less than 31.5%, the water content is not more than 13.93%, the total ash content is not more than 2.49% and the acid soluble ash content is not more than 0.19%. Test of chemical content of ethanol extract from the simplicia showed that chromatogram pattern with chloroform-ethyl acetate eluate (9: 1) showed Rf value 0.35, 0.39, and 0.36, for each region. The total phenolic content of ethanol extracts were 24.41%, 24.05% and 22.12%, respectively. The levels of α-mangostin in the ethanol extract of each region were 0.57%, 0.40%, and 0.25%.

Keywords: Simplicia, bark, ethanol extract, characterization, total phenol content, *Garcinia cowa* Roxb

1. Introduction

The use of traditional medicine in Indonesia has been going on since ancient times and traditional medicine has been used for generations. Generally traditional medicine is used to maintain health, prevent disease, treat disease, and restore health (Indonesia, 2000). West Sumatra is a fertile and rich region with various plants. Some of these herbs have been traditionally used by the community as medicinal substances.

Plants of the genus Guttiferae (Garcinia) lately much studied the content and activity. This genus is reported to contain xanthon, benzophenone, triterpen, bioflavonoid, benzoquinone, α-mangostin, cowanin, cowanol, cowaxanthon, rubraxanthon, β-mangostin, tetrapreniltolouquinon, and xanthon prenylated (Rukachaisirikul *et al*., 2008; Wahyuni *et al*., 2004; Matsumoto *et al*., 2003; Ali *et al*., 2000). The xanthon compound is primarily known for its potential as an anticancer (Jabit *et al*., 2009). Not only its potential as an anticancer, have some studies also mentioned that this xanthon compound also has potential as antimalarial and antibacterial. One of

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the plants in this genus began a lot of research is *Garcinia cowa* Roxb known as kandis or asam kandis. Garcinia cowa Roxb has been used for medicine by the people as antipyretic and anti-inflammatory.

This plant has long been used by people, especially for candied or seasoned cuisine or spices (Heyne, 1987). Kandis (*Garcinia cowa* Roxb) as one of the genera of Garcinia plants contains oxygenated and alkylated xanthon compounds, flavonoids and benzophenone in almost all parts of this plant (Ampofo *et al*., 1986). In addition to xanthon this species is also known to contain benzophenone and flavonoid compounds. This group of compounds is known to have various activities such as antimicrobials, antimalarial, antioxidants, anti-inflammatory, antitumor and anticancer agents (Komguen *et al*., 2005). The bark of kandis contains a variety of compounds, one of which is α-mangostin which is one of xanthon group compounds (Wahyuni, *et al*., 2004). It is also used as an antipyretic (na Pattalung *et al*., 1994). The fruits and leaves of this plant are used to improve blood circulation, as an expectorant, to treat coughs and have an effect on actively to overcome digestive disorders, but their roots can also be used to reduce fever (Poomipamorn & Kumkong, 1997).

Based on the above, the researcher is interested to do research about the characterization of simplicia and ethanol extract of bark from kandis (*Garcinia cowa* Roxb). This is because there is no research and supporting data about the characteristics of this stem bark. This study aims to look at the characteristics of each sample taken from different regions.

The simplicia characterization parameters tested are specific and nonspecific parameters. Specific parameters include organoleptic, microscopic, soluble compounds in a particular solvent, looking at chromatogram patterns, determining total phenolic content and determining levels of chemical content. Non-specific parameters include drying losses, total ash content and acid soluble ash content. The extract characterization parameters tested are specific and nonspecific parameters. Specific parameters such as organoleptic examination see chromatogram pattern, determination of total phenolic content and determination of chemical content. Non-specific parameters include calculation of yield, moisture content, total ash content and acid soluble ash content. Macroscopic observation involves direct observation of the plant. This plant is taken from three different areas namely Solok, Padang and Padang Pariaman.

2. Materials and Methods

2.1 Equipment

The tools used in this research are: Distillation apparatus, rotary evaporator, analytical scale, oven, storage rack, dropper, Erlenmeyer flask, 500 ml flask, condenser, funnel, measuring cup, measuring flask, micropipette, ultra lamp violet, silicate crucible, protective pellet, steam plate, water bath, reaction tube, clogged flask, TLC plate (Silica gel 60 F254), Whatmann filter paper No. 1, UV-Vis Spectrophotometer (Shimadzu 265).

2.2 Materials

2.2.1 Plant

The plants studied were Garcinia cowa Roxb obtained from Solok, Padang and Padang Pariaman areas. The plant parts used in this study were simplicia and ethanol extract from Garcinia cowa Roxb stem bark.

2.2.1 Chemicals

Ethanol 96%, Folin-Ciocalteu reagent, distilled water, methanol, sodium hydroxide, sulphuric acid, toluene, α-mangostin, gallic acid, ethyl acetate, chloroform. All chemicals are purchased from Merck.

2.3 Procedures

2.3.1 Identification of plant

Plant identification was undertaken to ascertain the type of plant used for the study. The identification was done at Herbarium of Andalas University, Biology Department, Faculty of Mathematics and Natural Sciences, Andalas University.

2.3.2 Sampling

The samples used for this study were bark of asam kandis (*Garcinia cowa* Roxb) taken from Solok, Padang and Padang Pariaman, West Sumatra.

2.3.3 Preparation simplicia bark of asam kandis (*Garcinia cowa* Roxb)

2.3.3.1 Collection of raw materials

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Stem bark from plant asam kandis is taken from the trunk each 2 kg of wet. Then the bark is collected and cleaned.

2.3.3.2 Cutting

The collected stem bark is then cleaned and cut into small pieces.

2.3.3.3 Drying and Smoothing

The bark is reduced in size and dried by air-dried at room temperature for seven days. After drying, the stem bark is smoothed with a grinder tool to obtain a powder of simplicia.

2.3.3.4 Storage

The simplicia powder is kept in a brown bottle tightly closed, protected from sunlight and humid air.

2.3.4 Examination of simplicia

The examination of simplicia of asam kandis is done in accordance with the Indonesian Herbal Pharmacopoeia (Indonesia, 2013).

2.3.5 Preparation of *Garcinia cowa* extract

The simplicia powder from the bark of the stem of *Garcinia cowa* was used 1000 g. One part of dry powder was added 10 parts of ethanol 70%. The powder is soaked for the first 6 hours with occasional stirring, then let stand for 18 hours. The obtained macerate is separated by filtration. This process is repeated two more times with the same type of solvent and the solvent volume as much as half the volume of the solvent on the first run. All the macerates are collected, then evaporated with a vacuum evaporator or a low pressure vaporizer until a viscous extract is obtained.

2.3.6 Examination of extract

The examination of extract of asam kandis is done in accordance with the Indonesian Herbal Pharmacopoeia (Indonesia, 2008).

3. Results and Discussion

3.1 Identification and sampling of plant

In this study the samples used were bark of asam kandis (*Garcinia cowa* Roxb) taken from Solok, Padang and Padang Pariaman areas based on different altitude of Solok (450 meters above sea level), Padang (255 meters above surface sea) and Padang Pariaman (<100 meters above sea level). Samples taken from Solok area are in Tanjuang Alai area, from Padang area in Limau Manis area, and from Padang Pariaman area is in Sikapak area. The purpose of sampling from these three areas is to look at the characteristics of the bark of the kandis acid from each region. The sample was first identified in Herbarium Andalas, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University. The identification results show that the sample is true of *Garcinia cowa* Roxb. So the sample can be used for further research. The sample identification aims to provide an objective identity of the specific name of the sample (Indonesia, 2000). This is also because of this type of asam kandis more than one. The method used in the characterization of simplicia and extract refers to the Indonesian Herbal Pharmacopoeia (Indonesia, 2008).

3.2 Preparation simplicia bark of asam kandis (*Garcinia cowa* Roxb)

The bark taken and collected is cleaned first, then cut into small pieces and dried. The drying process is done by air dried. This process aims to keep the simplicia and chemical content contained in it not easily damaged and can last long. The dried sample was smoothed with a grinder to obtain a slightly coarse powder. Powder smoothing was aims to enlarge the surface area of the simplicia particle so that the area in contact with the solvent will be greater so as to facilitate the penetration of the solvent into the simplicia and multiply the drawing of the compounds contained in the simplicia with the solvent used.

3.3 Examination of simplicia

The examination of simplicia stem bark was done macroscopically by observing the physical form of the bark of *Garcinia cowa*. It aims to determine the characteristic of the stem bark of *Garcinia cowa*. The results show that *Garcinia cowa* has a rough, hard and dark brown bark. By organoleptic, the simplicia has a distinctive odour, a coarse powder and a biting taste.

The specific parameter of simplicia included in the characterization of simplicia is a microscopic test. The results show the identification fragment of the simplicia powdery bark of *Garcinia cowa*. The fragments can be

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seen under a microscope. The simplicia powder has an identifiable fragment consisting of cover hair, carrier beam, stem epidermis, sclerenchyma fibres, cortical parenchyma, and oil cells. Microscopic testing aims to determine the fragment identifier in the form of cell or plant tissue contained in simplicia bark stem of asam kandis which will be used for the characterization of simplicia, so as to prevent forgery of the simplicia. Microscopic examination of the simplicia powdery bark of *Garcinia cowa* is done by using 400 x enlargements which is aided by using chloral hydrate (see Figure 1).

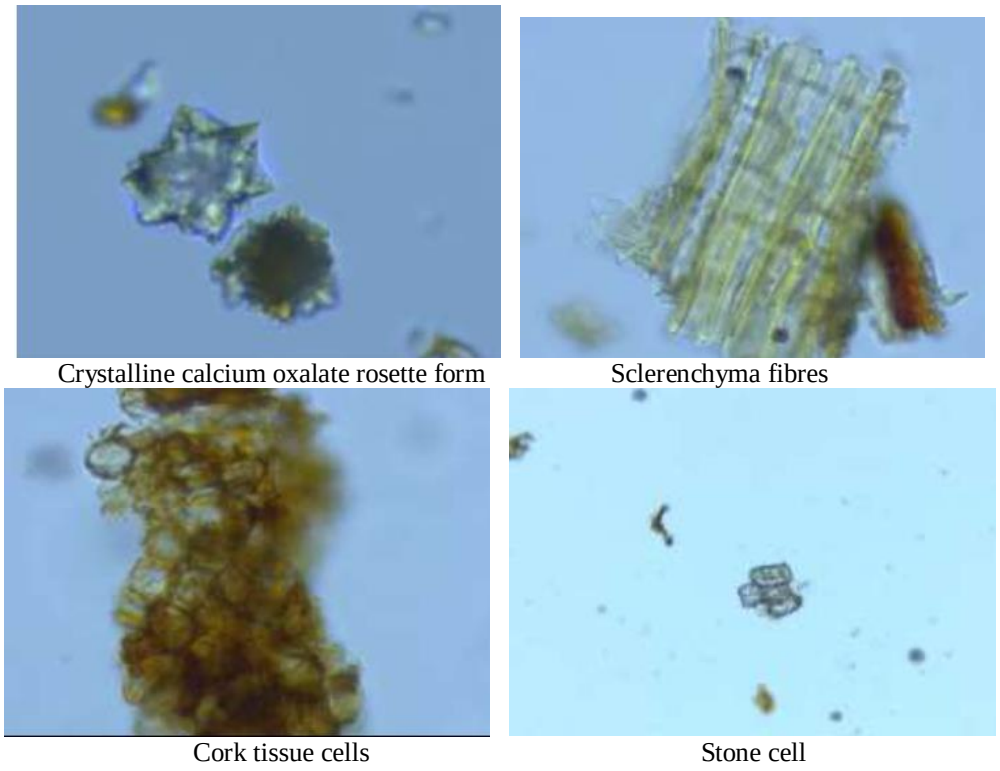

Figure 1: Fragment and microscopic test of simplicia stem bark of *Garcinia cowa* Roxb using a 400 times enlargement

The other specific parameters tested on the simplicia are parameters of soluble extract content in certain solvents, i.e. ethanol and water solvents. Both of these solvents are solvents that are allowed and qualify for pharmaceuticals. Determination of levels of soluble compounds in water and ethanol aims to obtain information on how much the content of active compounds that are polar (water soluble), and semi-polar active compounds (soluble ethanol) that can be extracted. The use of a water solvent is intended to dissolve the polar compound and the ethanol solvent to dissolve the less polar compound present in the simplicia (Saifudin *et al.*, 2011). The result of determination of water soluble extract level from simplicia bark of *Garcinia cowa* showed that for each region of 11.91% ± 0.42 (Solok), 15.10% ± 0.45 (Padang) and 11.71% ± 0.69 (Padang Pariaman). The content of soluble ethanol extract for each area is 18.72% ± 0.92 (Solok), 19.43% ± 1.13 (Padang) and 16.59% ± 0.42 (Padang Pariaman).

The drying shrinkage parameter was tested by measuring the remaining substance after drying at 105 °C for 30 minutes or until constant weight. The drying shrinkage parameters are tested with the aim of providing maximum limits on the amount of lost compounds in the drying process. Value or range obtained related to purity and contamination (Indonesia, 2000). The drying losses obtained from the bark of *Garcinia cowa* for each region were 7.98% ± 0.11% (Solok), 7.22% ± 0.30% (Padang) and 7.32% ± 0.07% (Padang Pariaman).

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3.4 Preparation of *Garcinia cowa* extract

Simplicia in fine form is then extracted by using the maceration method. The maceration method is chosen because it is a simple way of sifting where the solvent will penetrate the cell wall and into the cell cavity containing the active substance (Voigt, 1994). In addition, maceration can also be used for simplicia that have active compounds that are not resistant to heating. The extraction process in which one part of the simplicia is immersed in ten parts of 75% ethanol solvent for 6 hours with occasional stirring and then stays for 18 hours. Soaking the sample is done with two repetitions. Immersion of the sample is performed in a place that is protected from light to avoid decomposition of substances that are less stable to light. The solvent used is 70% ethanol. The advantage of using ethanol solvent is that it is universal enough to dissolve almost all secondary metabolites present in the simplicia. Ethanol can also precipitate proteins and inhibit the action of enzymes so that active substances can avoid hydrolysis and enzyme oxidation (Harbone, 1987). In addition, ethanol is also more economical because its price is cheaper and its toxicity is much lower than other solvents (Indonesia, 2000).

The resulting ethanol macerate is then combined and concentrated using a rotary evaporator in a vacuum. It is intended to lower the air pressure on the surface of the macerate so that it will lower the boiling point and the solvent can evaporate faster so that no high temperature is needed to evaporate the solvent. This can reduce the likelihood of decomposition of the active substances that cannot stand with higher heating temperatures. The remaining solvent when present is evaporated over the water bath until the weight of the extract becomes constant. The specific parameter for the extract is the organoleptic observation. This observation indicates that the extract is dark red, distinctive smell and has a bitter taste and a viscous extract. The ethanol extracts obtained from Padang, Solok and Padang Pariaman were 399 g, 315 g, and 360 g, respectively, with yields of 39.9%, 31.5%, and 36%, respectively. Determination of this yield is aimed to know the amount of simplicia needed to manufacture a certain amount of viscous extract. The results of this yield may also indicate the possible number of chemical compounds contained in the extract and show the maximum amount of solvent used for the extraction process.

Furthermore, the observation of non-specific parameters is total ash content of simplicia and bark extract of *Garcinia cowa*. Simplicia bark of *Garcinia cowa* showed total ash content of each region of 5.09% ± 0.48 (Solok), 4.65% ± 0.46 (Padang) and 6.66% ± 0.26 (Padang Pariaman). Stem bark extract showed per cent ash content for each region of 2.49% ± 0.06 (Solok), 1.67% ± 2.39 (Padang) and 1.5% ± 0.05 (Padang Pariaman). Ash is a residual inorganic substance of combustion of an organic material. The content of ash depends on the type of ingredients and the method of feeding. The ash content has something to do with minerals of a material which can be either organic or non-organic salt. The minerals present in a material may be of two kinds of salts, e.g. organic salts, for example, salts of malic acid, oxalate, acetate, etc. and inorganic salts, e.g. phosphates, carbonates, chlorides, nitrate, sulphates and alkali metals. In addition to these two salts, sometimes minerals can form as complex compounds that are organic. Determining the amount of minerals in its original form is very difficult. Therefore, it is usually done by determining the residual burning of the mineral salts known as spoilage (Sudarmandji *et al.*, 1986). Determination of total ash content is intended to provide an overview of internal and external mineral content derived from the initial process until the formation of the extract. In this process the extract is so permitted that the organic compounds and their derivatives are destructible and evaporate to leave only inorganic and mineral compounds alone.

The acid insoluble ash content was obtained from the total ash content and dissolved in 2N hydrochloric acid to evaluate the extract on the contamination of silica-containing materials such as soil and sand. The acid insoluble ash content for the simplicia in each region is 0.14% ± 0.13 (Solok), 0.21% ± 0.02 (Padang), and 0.13% ± 0.06 (Padang Pariaman). The acid insoluble ash content for bark extract of each area was 0.19% ± 0.09 (Solok), 0.05% ± 0.03 (Padang), and 0.08% ± 0.06 (Padang Pariaman).

The non-specific parameter for the extract is the moisture contained in the extract. The result of measurement of water content from each region is 19.79% ± 4.17 (Solok), 12.60% ± 2.29 (Padang) and 9.26% ± 3.01 (Padang Pariaman). Determination of moisture content is done by the method of distillation. The purpose of this parameter is to limit the water content in the material (Ministry of Health, 2000). High water levels will grow fungus that is not good for health. Measurement of moisture content in a material is necessary in many fields, moreover on a plant extract. The measurement of moisture content is also done to maintain the quality

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of the extract (Soetarno & Soediro, 1997). The moisture content depends on the type of extract, e.g. the moisture content of the viscous extract is 5-30% (Voigt, 1994). This moisture content is a non-specific parameter that is not directly related to pharmacological activity but affects the security and stability aspects of the extract and the resulting preparation.

The simplicia and extract chromatography patterns were performed by thin layer chromatography (TLC). The solvent used was chloroform: ethyl acetate (9: 1, v / v) and as comparator used α-mangostin. Stain spots on the TLC can be seen under UV light with a wavelength of 254 nm. The chromatogram showed that the Rf values for the simplicia of Solok, Padang and Padang Pariaman areas were 0.39, 0.39, and 0.37, respectively. While the Rf value for the ethanol extract of the stem bark from Solok, Padang and Padang Pariaman areas were 0.35, 0.39, and 0.36, respectively. The above TLC pattern shows that on the bark of Garcinia cowa there are α-mangostin compounds.

The method of determining total phenolic content using Folin-Ciocalteu reagent is a simple and sensitive method for phenol compounds and reagents used in small amounts. The phenol reagent is yellow and will change colour to dark blue when reacted with an extract solution which has been added with sodium carbonate solution. This blue complex solution will be determined its absorbance value with a visible light spectrophotometer with a wavelength of 730 nm so that the content of phenolic compounds of the sample can be known.

Absorbance of gallic acid after addition of Folin-Ciocalteu reagent can be seen in Table 1. The absorbance is measured at 730 nm wavelength. The calibration curve can be seen in Figure 2.

Table 1: Absorbance of standard solution of gallic acid + Folin-Ciocalteu reagent at a maximum wavelength of 730 nm with a spectrophotometer

The concentration of standard gallic acid solution (µg/mL)	Absorbance
30	0.275
40	0.335
50	0.442
60	0.554
70	0.654

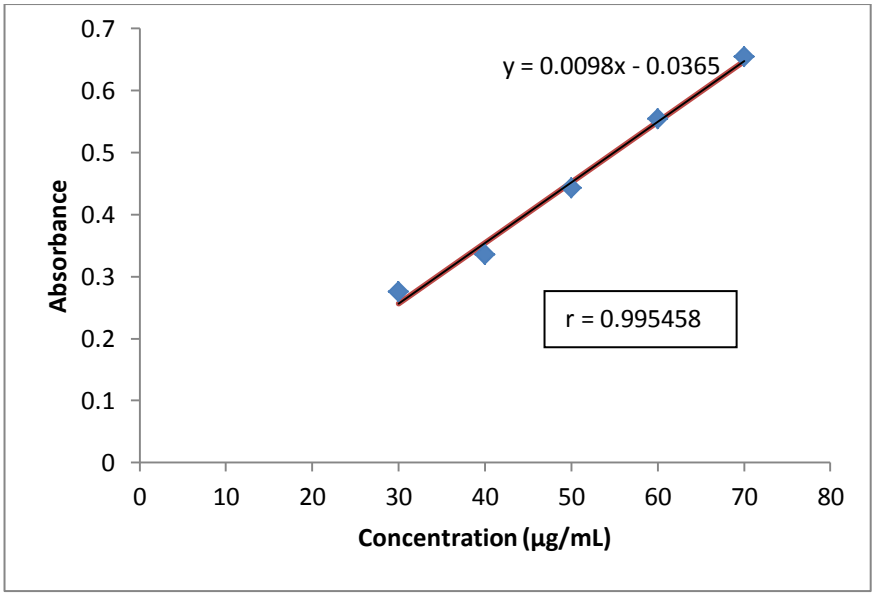

Figure 2: Calibration curve of gallic acid solution and Folin-Ciocalteu reagent

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In the determination of total phenolic compound content as comparative solution used was gallic acid. The wavelength used for the absorbance measurement is 730 nm. This is based on the reference book in this study that is Pharmacopoeia Herbal Indonesia. Based on the measurements, the total phenolic content of the simplicia from each region was 11.4% (Solok), 9.75% (Padang) and 9.78% (Padang Pariaman). The total phenolic content in the extract from each sample was 24.41% (Solok), 24.05% (Padang) and 22.12% (Padang Pariaman).

Determination of the content of a-mangostin compounds in simplicia and extract aims to obtain the total levels of certain metabolite groups thought to have pharmacological activity. The method used for determining the levels of these compounds is thin layer chromatography - densitometry. The area under the curve of thin layer chromatography - densitometry for α-mangostin at various concentrations is shown in Table 2 and the calibration curve is shown in Fig. 3. Based on the results of the research, it was found that the levels of α-mangostin in the bark of *Garcinia cowa* were 0.0597% (Solok), 0.0461% (Padang) and 0.0416% (Padang Pariaman), respectively. The levels of α-mangostin contained in the bark extract of *Garcinia cowa* were 0.57% (Solok), 0.40% (Padang) and 0.25% (Padang Pariaman), respectively.

Table 2: Area under curve of α-mangostin by thin layer chromatography – densitometry

Concentration(µg/mL)	Area under curve (AUC)
50	2107.4
100	4445.5
250	9364.3
400	14760.3

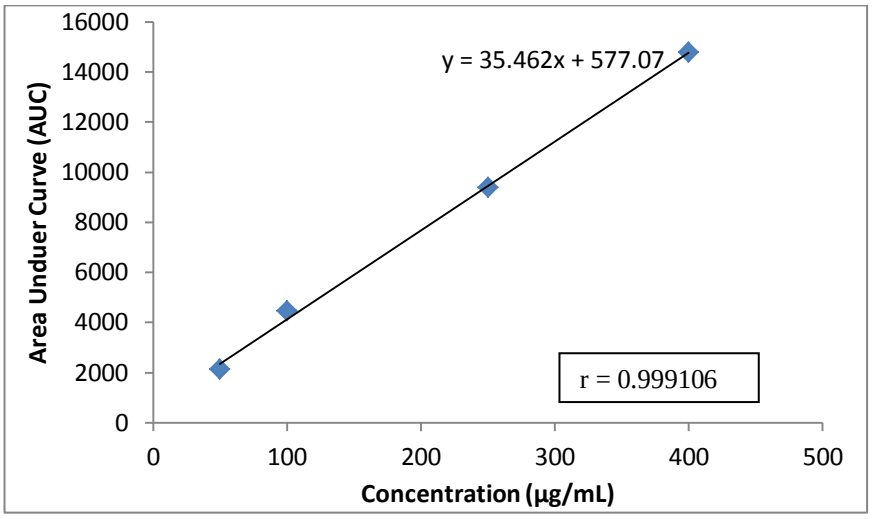

Figure 3: Calibration curve of α-mangostin by thin layer chromatography - densitometry

Determination of the chemical content indicates that the amount of chemical content possessed by the simplicia and extracts of the three regions has significantly different levels of chemical content. This is due to the difference in altitude of the sampling site used. The plateau is a vast plain located in the hills and lowlands is a flat and broad ground with an altitude of less than 200 m upper sea level, commonly found around the coast.

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Garcinia cowa grown in Solok, Padang and Padang Pariaman area have temperatures ranging from 12.50 ^{o}C - 24.60 ^{o}C. Character of *Garcinia cowa* is expected to be a parameter for the drug industry in the manufacture of quality herbal medicines.

4. Conclusion

4.1 Character of simplicia bark of *Garcinia cowa*

The macroscopic test of bark simplicia Garcinia cowa showed that the simplicia was a rather coarse powder, dark brown, distinctive odour, and chelate taste. This microscopic simplicia test shows the presence of identical fragments of calcium oxalate crystals of rosette form, cork tissue, sclerenchyma fibres. This physicochemical parameter shows a drying rate of not more than 10%, total ash content of not more than 6.66%, acid soluble ash content not more than 0.21%, water soluble essence not less than 11.71%, soluble essence ethanol is not less than 16.5%. Chromatogram pattern with chloroform eluent: ethyl acetate (9: 1) obtained Rf value for each region with α-mangostin comparator that is 0.39, 0.39, and 0.38. Total phenolic compound content for each region was 11.44%, 9.75%, 9.78%. The levels of α-mangostin for each region were 0.06%, 0.05%, 0.04%.

4.2 Character of *Garcinia cowa* bark extract

The ethanol extract of Garcinia cowa stem bark is in the form of thick extract, dark red colour, distinctive smell and bitter taste. The yield is not less than 31.5%, the moisture content is not more than 13.93%, the total ash content is not more than 2.49% and the acid soluble ash content is not more than 0.19%. Chemical content test included chromatogram pattern with chloroform eluent: ethyl acetate (9: 1) showed Rf value for each region with α-mangostin comparator of 0.35, 0.39, and 0.36. Levels of total phenolic compounds extract for each region were 24.41%, 24.05%, 22.12%. The levels of α-mangostin for each region were 0.57%, 0.40%, 0.25%. The research shows that the simplicia and extract from Garcinia cowa taken from three regions with different height of place have different character and chemical content.

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