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Isolation of the main chemical compound from dichloromethane extract of kandis acid stem leather (*Garcina cowa* Roxb.).

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ABSTRACT

Isolation of main chemical compound from dichlorometane extract of G. cowa's stem bark has been done. The ground air dried stem bark (2 kg) was given 20,3 grams thick extract of n-hexane and 76,76 grams thick extract of dichlorometane. Separation was conducted by colomn chromatography and radial chromatography. The purification was carried out trough crystallisation. FM was isolated as yellow needle crystal (1,013), with Rf value 0,55 and melting point at 207-209 oC. On other hand, DM was also obtained 132 mg of yellow amorphous compound, with Rf 0,75 and melting point at 218-220 oC. The rendemen of FM is 2,53%, while for DM is 0,33%. Based on Melting point, thin layer chromatography profile using standard compound, ultraviolet and Infrared spectra, FM is identified as rubraxanthone.

Keywords: *Garcinia cowa* Roxb., dichlorometana.

INTRODUCTION

Natural ingredientsin Indonesia is one of the seven countries with the greatest biodiversity, this fact certainly has potential in the development of herbal medicine based on medicinal plants in an attempt to self-reliance in the health sector [1]. The use of traditional medicine and medicinal plants in Indonesia has been going on since ancient and traditional medicine has been used for generations. Traditional medicine is generally used to maintain health, prevent disease, cure disease, and restore health [2].

One of the many plants studied are plants of the genus Garcinia, with Garcinia species cowa Roxb. From various studies that have been done, it is known that Garcinia cowa Roxb. containing xanton, xanton prenylated, nor xanton tetraoxygenationed on almost all parts, such as roots, stems, bark, leaves, fruit, and sap [3].

Based on the test bioactivity, Garcinia cowa Roxb. show pharmacological activity as an anti-HIV, anti-cancer, anti-inflammatory, antitumor, the treatment of hepatitis, colitis, antileukimia [4], or free radical antioxidant [5], and also have a cytotoxic effect [6].

According to Darwati, n-hexane extract of the bark of Garcinia cowa Roxb. produces a compound xanton types xanton tetraoxygenationed namely kowanin [7].Another study conducted by Wahyuni showed that n-hexane extract of bark Garcina cowa Roxb. obtained compound [2E, 6E, 10E] - (+) - 4 β-hydroxy-3-methyl-5 β- (3,7,11,15-tetramethyl-2,6,10,14- hexadecatetraenyl-2-Cyclohexene -1-one. In the ethyl acetate fraction of compounds found 6-hidroxycalabaxanthone, 2 - (- methyl-2butenyl) -1,5,6-trihydroxy-3-methoxy-4- (1,1-dimethyl-2-propenyl) -9H-xanthen-9- one, rubraxanton, cowanin, α -mangostin, and 1,3,6-trihydroxy-7-methoxy-4- (acetoxy-3-methyl-2-butenyl) -8- (3,7dimethyl-2,6-octadienyl) xanthones [8].

The test of cytotoxic effects on breast cancer cells T47D of four types of results fractionation of the stem bark of Garcinia cowa Roxb. has been conducted, that extracts nheksana, dichloromethane, ethyl acetate, and ethanol thus

obtained dichloromethane extract as an extract of the most active of potentially cytotoxic against breast cancer cells T47D with IC50 4 mg / mL. Dichloromethane of stem bark kandis acids extract also have spurred activity in T47D breast cancer cell death through apoptosis mechanism, so the potential to be developed as chemopreventif and anticancer [9].

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Based on this, the study was conducted in an attempt to isolation of the main chemical compounds of dichloromethane of stem bark kandis acid extract (Garcinia cowa Roxb.). This study was also conducted in order optimizing method for obtaining primary chemical compound, which the market has a high enough price. The method that will be used to isolate chemical components of the plant by maceration, examination by thin layer chromatography, column chromatography separation and purification by recrystallization. The characterization of isolated compounds include organoleptic examination, chemical examination, the determination of the melting range, thin layer chromatography inspection, ultraviolet-visible spectrophotometer, and an infrared spectrophotometer.

MATERIALS AND METHODS

Materials and Equipment

The tools are used: a set of tools maceration, rotary evaporator, pumpkin rotary, column chromatography, a set of tools kromatotron, beakers, erlenmeyer, test tubes, test tube rack, the plate drops, pipette, pipette capillary, chamber, vial, tweezers, spatula, analytical weigher, lights UV254nm and UV365nm, John Fisher Melting Point Apparatus, ultraviolet spectrophotometer, an infrared spectrophotometer.

The materials used: stem bark acid kandis, filter paper, cotton, aluminum foil, aquadest, metalic magnesium, concentrated hydrochloric acid, concentrated sulfuric acid, sulfuric acid 2N, acetic anhydride, iron (III) chloride, reagent Mayer, reagent vanilin -sulfat reagent Liberman Burchard (LB), chloroform, chloroform, ammonia, methanol, ethyl acetate, n-hexane, dichloromethane, silica gel 60 and plate silica gel 60 F254.

Sampling

Samples in the form stem bark kandis acid 5kg taken in Batu Busuk, Limau Manis, Padang, West Sumatra.

Identification of Samples

Identification of the samples was done at the Herbarium of Andalas University Padang (ANDA).

Extraction and Fractionation

5 kg of fresh bark kandis acid cleaned of impurities, then cut into pieces and dried in the sun. Then stem bark dried grinded to a powder. After grinded, extracted by maceration method stratified. First sample macerated by using hexane for two days and take the macerate then be evaporated with a rotary evaporator, then again using hexane to macerate the dregs with the same treatment until macerat looks clear. After that is done maceration using dichloromethane with the same treatment with hexane.

Isolation and Purification of Compounds

40 g of extract viscous fraction dichlorometane readsorpted with silica gel 60 with the same tightly before it is put into the chromatographic column. Then chromatographed silica gel column by using as many as 400 g. Silica slurry made using solvent nheksana. After the column is ready, put silica slurry and powder samples preadsorbsi sown into the upper surface of the silica slurry tealh in the column. Column chromatography eluted using a solvent in which the polarity is increased in stages (step gradient polarity / SGP).

n-Heksana ⟶ 100 %

n-Heksana : etil asetat ⟶ 9 : 1

n-Heksana : etil asetat ⟶ 8 : 2

n-Heksana : etil asetat ⟶ 7 : 3

n-Heksana : etil asetat ⟶ 6 : 4

n-Heksana : etil asetat ⟶ 5 : 5

n-Heksana : etil asetat ⟶ 4 : 6

Etil asetat ⟶ 100%

Metanol ⟶ 100%

The results column accommodated with 100 mL infusion bottle then monitored by TLC. TLC results can be seen with a UV lamp λ254 or λ365 and stain the same pattern can be combined. Having accommodated obtained subfraksi A, B, C, D, E, F, G, H, I and J.

Furthermore, the separation of compounds made against subfraction F. The preparation process for the same chromatographic column saperti done before. Namum, column chromatography eluted using a solvent in which the polarity is not raised in stages (isocratic). The solvent used is n-hexane: ethyl acetate (9: 1). Subfraction with the same TLC pattern combined. Then recrystallized.

Characterization of Compound Results Isolation

Isolated compounds obtained were characterized as follows:

1. organoleptic examination done visually observing the shape and color of isolated compounds.
2. Determination of the melting distance determination within the melting is done by means of Fisher John Melting Point Apparatus in Biota Laboratories Sumatra Andalas University in Padang.
3. UV-Vis Spectrophotometer UV spectrum examination carried out by using UV-Vis spectrophotometer. Isolated compounds dissolved in methanol, then measured absorbance.
4. Examination of the infrared spectrum IR spectra were measured using FT-IR spectrophotometer Perkin Elmer. A number of 1 mg sample is then measured absorbance.

RESULTS

1. Examination of the chemical constituents of skin badang Garcinia cowa Roxb. showed a class of flavonoids, terpenoids, steroids, saponins and phenolic.
2. From 2 kg samples of dried stem bark of Garcinia cowa Roxb. viscous extract obtained n-hexane 20.3 grams and 76.76 grams dichloromethane extract thick.
3. From the dichloromethane extract obtained two compound FM and DM with the specifications:

a. FM: yellow colored crystalline needles as much as 1,013 grams, which have Rf 0.55 eluted with n-hexane: ethyl acetate (6: 4). It melts at a temperature of 207-209 ° C, very soluble in ethyl acetate and acetone, and insoluble in nheksana. UV spectrum in methanol shows a maximum absorption at a wavelength of 241.2 nm (Abs = 0.737). The infrared spectrum showed strong absorption in the area of wave number 3424.62 cm-1; 2916.30 cm-1; 1605.79 cm-1; and 1159.83 cm-1.

b. DM: yellow colored amorphous as much as 132 mg that has Rf 0.75 eluted with n-hexane: ethyl acetate (6: 4). It melts at a temperature of 218-220 ° C UV spectrum in methanol shows a maximum absorption at a wavelength of 241.6 nm (Abs = 0.371). The infrared spectrum showed strong absorption in the area of wave number 3362.97 cm-1; 2915.37 cm-1; 1427.82 cm-1; and 1294.60 cm-1.

1. Results rendemen compound obtained from the extracts dikolom:

a. Compounds FM namely: 2.53%

Compounds DM ie: 0.33%

DISCUSSION

The process of isolation begins on the extraction of stem bark kandis acid. The bark is cleaned of impurities, then cut into pieces and dried in the sun. Then the dried bark grinded until smooth. This smoothing aims to enlarge the surface area of the sample, so as to optimize the extraction process due to extensive contact with the sample solvent becomes larger and eventually will facilitate the process of extracting the compounds contained in the sample tissue. After sampling grinded, then performed the extraction process. Extraction of the main chemical constituents of the stem bark kandis acid fractionation is done by maceration method. Maceration chosen because it can be used for the extraction of samples in large quantities, does not require any special treatment, the process is easy, too simple equipment needed and the absence of heat during the extraction process can anticipate damage to compounds that are thermolabile.

In the maceration process, the solvent used is n-hexane and dichloromethane. Use of these solvents are nonpolar solvents starting from the sequence of n-hexane and dichloromethane. Immersion bark kandis acid samples to the solvent is done in a pumpkin percolator. Beginning with the most nonpolar solvents are n-hexane, directly intended to separate compounds based on the level of the polarity at the start of the nonpolar compound contained in the sample to be drawn perfectly solvent. Stirring is done to accelerate the penetration of the solvent into the sample so that the chemical components in it quickly dissolved. Maceration process is carried out in a protected area of light to avoid possible degradation of the compound structures especially for nonpolar and other compounds that are less stable because of the light. Results maceration removed and filtered with cotton every two days and this process is repeated up to seven repetitions. After seven repetitions, the pulp is dried to its next maceration using dichloromethane and treated the same as the previous maceration process.

The extract n-hexane and dichloromethane were obtained evaporated using distillation in a vacuum. This process will be faster to evaporate the solvent, wherein the solvent vapor pressure becomes lower so that the solvent can evaporate at a lower point than the boiling point of the original. It is useful to minimize damage thermolabile compounds contained in the sample. And do concentration by rotary evaporator to obtain an extract obtained thick and heavy n-hekasana extract as much as 22.3 g and dichloromethane extract as much as 76.76 grams.

Purification of the compounds performed on dichloromethane extract. Beginning with using column chromatography on silica gel gravity 60 as the stationary phase. Elution system used in this method is the elution system with increased polarity in stages (step gradient polarity / SGP). Elution system have been because they have not known solvents for better separation of compounds.

The column was prepared by making a slurry of silica (silica gel 60) using n-hexane. Samples are prepared by preadsoprtion whereby the sample is dissolved in a solvent that dissolves and is mixed with silica gel in the number of samples and silica ratio of 1: 1 and then evaporated the solvent until completely dry. Silica slurry that was created entered into a column of silica gel while being hit in order to condense. Samples were prepared inserted into the chromatography column and then the column was eluted with mobile phase was increased in increments

Column accommodated in 100 mL vials and each subfraction monitored TLC pattern under UV254 lights. Subfraction with the same stain combined. Subfraction obtained is subfraction A (1.85 grams), subfraction B (0.195 grams), subfraction C (0.436 grams), subfraction D (2.13 grams), subfraction E (6.057 grams), subfraction F (3,12 gram), subfraction G (4.60 grams), subfraction H (5.356 grams), and subfraction I (3.271 g). Then monitored as well as patterns of each subfraktion the TLC.

Column chromatography followed on subfraktion F, because of the results of monitoring subfracrtion TLC shows the desired target compound. Column chromatography is done in the same way. Elution system used is a system with polarity storey elution (isocratic) using the mobile phase n-hexane: ethyl acetate (9: 1). Elution systems have been selected for chemical components in a fraction can be split with well known of stain patterns on TLC. Results column accommodated in a 10 mL vial and subfraction with the same stain combined. It was found that nine subfraction are subfraction F1, F2, F3, 4, F5, F6, F7, F8, and F9. From the results of the TLC monitoring, subfraction F7 show the stain with a little impurity.

Fm pure compound (102 mg) was obtained from subfraction sixth column chromatography results after recrystallized. Recrystallization process is a process of purification of the compounds in the form of crystals. The process is based on the difference in the solubility of a substance in hot and cold solvent by using two solvents. Selection of solvent based on the difference in solubility of the compound in a second solvent. Solvents that one should be able to dissolve the desired compound and other solvent that can not dissolve the compound. Solvents used in the recrystallization process are ethyl acetate and n-hexane. Subfraction F7 gained as much as 1,666 grams.

Recrystallized several times using a suitable solvent in order to obtain the pure compound which showed a stain round when monitored with TLC plate.

In subfraktion G also done chromatography column, because of the results of monitoring TLC in the first column. Subfraction G had a stain pattern similar to subfraction F. Column chromatography is done in the same way. Obtained eight subfraction, namely subfraction G1, G2, G3, G4, G5, G6, G7 and G8. From the results of the TLC monitoring, subfraction G5 (1,264 grams) shows the stains.

Fm pure compounds and compounds G5 combined for TLC results that show a pattern of stains that formed after the monitored with TLC plate. Then be purified by recrystallization using ethyl acetate and n-hexane to obtain the pure compound FM (figure 1). FM pure compound is obtained in the form of yellow colored needle crystals weighing 1.013 grams. Test results show that the melting range of this compound melts at a temperature of 207-209 ° C. Examination of the UV spectrum in methanol shows absorption at a wavelength of 241.2 nm and 311.00. Based on the literature λmaks absorption at 241 and 312 indicate a xanthone core (Figure 2) (Wahyuni, et al., 2015).

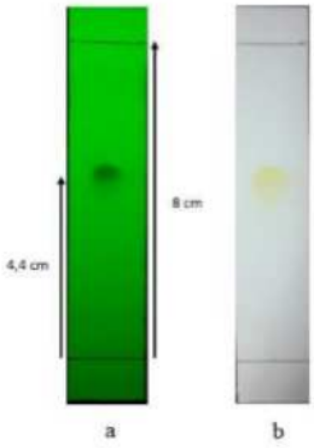

Figure 1 : TLC profile FM compounds

a. Under UV 254 lights
b. Using steam iodine stain seer

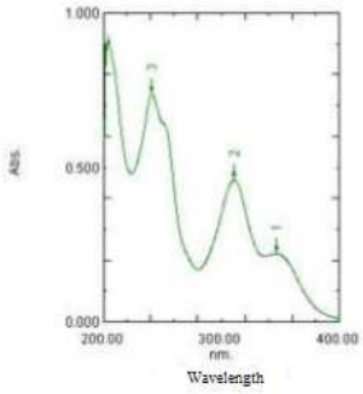

Figure 2: UV-Vis spectra of compounds FM

On examination of the infrared spectrum showed the compound FM has strong absorption in the area of wave number 3424.62 cm-1 is suspected of strain O-H; 2916.30 cm-1 probably derived from buckling aromatic C-H; 1605.79 cm-1 probably derived from stretching C = O; and 1159.83 cm-1 probably derived from helping C-O (Figure 3).

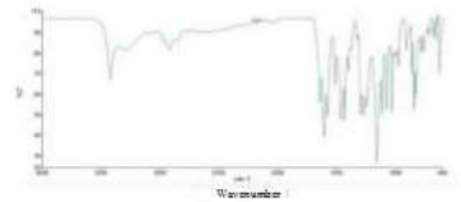

Figure 3: IR spectrum of compounds FM

UV spectrum, IR spectrum, TLC profile and FM melting distance is compared with the comparison compounds rubraxanton. The results showed the UV spectrum, the maximum absorption FM identical to the comparator compounds rubraxanton. The results also show the IR spectrum, FM wave number is identical to the comparator compounds rubraxanton (Attachment 16). In addition, the profile data rubraxanton TLC pure by GC-FM show the same Rf (Attachment 11) and FM melting distance compared to the distance melting compound rubraxanton comparison shows the distance melting compound is equal to the distance rubraxanton melting (207-209 ° C). From this data it is concluded that the compound FM is rubraxanton.

Isolation is also done on the chemical content subfraction D. isolation process steps starting compounds of column chromatography with the phase of motion n-hexane: ethyl acetate (9: 1) to obtain five subfraction, namely subfraction D1, D2, D3, D4, and D5. Having monitored by TLC Subfraction D2, D3 and D4 are combined and performed again by column chromatography separation so as to three subfraction (D-I, D-II and D-III).

In subfraction D1 is separated by using kromatotron. Separation using kromatotron have been selected for the TLC results showed two patterns stain the meeting. Separation in this way is faster and solvents used are also less than in the chromatography column. Namely The stationary phasesilica gel coated on a quartz glass plate and used for the mobile phase dichloromethane 100%. Mobile phase have been selected for the chemical components in the sample can be split with well known from the TLC stain patterns.

Things to do before construction kromatotron is sample preparation. Samples were dissolved with eluent used as mobile phase and etched by using a pipette slowly into the hole where the mobile phase flow, then the new eluent flow into the hole through the pump piston. To determine the course of the elution process is monitored by a UV lamp. Results kromatotron accommodated in a 10 mL vial and each subfraksi monitored TLC pattern under UV light. Subfraction with the same stain combined. Obtained two subfraction namely D and D-a-b.

Subfraction D-I, D-A, and D-b are combined then performed again kromatotron purification by using a mobile phase of dichloromethane 100%. The results of monitoring of patterns TLC showed a stain pattern that forms after eluted with an appropriate mobile phase.

DM pure compounds obtained in the form of yellow colored needle crystals weighing 132 mg. Test results show that the melting range of this compound melts at a temperature of 218 220 ° C (Figure 4)

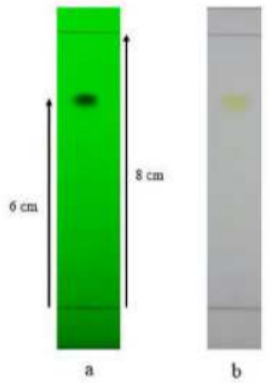

Figure 4: Profile KLT compound DM
(A) Under a UV lamp 254 (B) Using steam iodine stain penampak

Examination of the UV spectrum in methanol shows a maximum absorption at a wavelength of 241.6 nm (Abs = 0.371) (Figure 5). Based on the maximum absorption band obtained indicated the existence of conjugated double bonds, because these conjugate systems absorb light at wavelengths above 200 nm and indicates a chromophore which provides transition of π to π *.

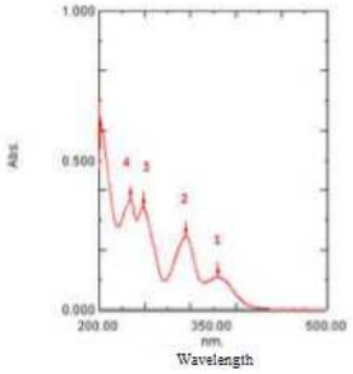

Figure 5: UV-Vis spectrum of compounds DM

On examination of the infrared spectrum showed the compound DM has strong absorption in the area of wave number 3362.97 cm-1 is suspected of strain O-H; 2915.37 cm-1 probably derived from buckling aromatic C-H; 1427.82 cm-1 (CH3); and 1294.60 cm-1 is suspected of helping the C-O (Figure 6).

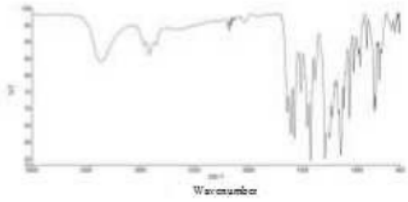

Figure 6: IR spectrum of compounds DM

CONCLUSION

1. From the 2 kg samples of dried stem bark of Garcinia cowa Roxb. n-hexane extract obtained 20.3 grams and 76.76 grams of dichloromethane extract.
2. From the dichloromethane extract obtained main chemical compound FM as much as 1,013 grams. TLC of the profile data with the comparison compound, melting range, the spectrum of UV and IR spectra can be concluded that the compound FM is rubraxanton.
3. From the dichloromethane extract obtained compounds as much as 0.132 g DM. Distance mp 218-220 ° C. UV spectrum of in methanol shows a maximum absorption at a wavelength of 241.6 nm (Abs = 0.371). The infrared spectrum showed strong absorption in the area of wave number 3362.97 cm-1; 2915.37 cm-1; 1427.82 cm-1; and 1294.60 cm-1.

SUGGESTION

It is suggested to further researchers to conduct structure elucidation of compounds DM and conduct research on the pharmacological activities of these compounds.

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