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Isolation and Identification of Free Radical Scavenging Compound from Stem Bark Soursoup (*Annona muricata* L.) Extract

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Abstract

Isolation and Identification of free radical active compound from stem bark soursoup (Annona muricata L.*) extract. The aim of this research is to observe the chemical compound of stem bark the soursoup (A. muricata* L.*) which is active as a free radical. 550 gram of stem bark the soursoup (A. muricata* L.*) was by graduate extracted using n-heksan, ethyl acetat and ethanol solvents respectively. Extract result is the extraction, it was produced 2.4 gram n-heksan extract, 8.4 gram ethyl acetat extract, 2.1 gram ethanolic extract. The isolation of ethyl acetat extract was conducted using colom chromatography (TLC) method using eluent n-hexane : ethyl acetat (7:3) and it was produced four fractions. The third fraction was isolated using preparative thin layer chromatography with mob ile phase n-hexane : ethyl acetat (6:1) and got 2 bands. The isolate identified by UV-Vis spectroscopy, IR spectroscopy and analyzed by chemistry reaction. From the data result show that isolate is acetogenin.*

I. Introduction

In everyday life, we cannot be free from free radical compounds. The cigarette smoke, fried and baked foods, certain drugs, toxins and air pollution are some sources of free radical formation compounds (Li et al., 2019). Lately many degenerative diseases such as cancer, heart disease, arthritis, diabetes, liver, and others (Argoff, Bhullar, & Galluzzi, 2019).

This degenerative diseases caused by free radicals in the body is not able to neutralize the increase in the concentration of free radicals (McArdle & Jackson, 2019). One of the plants used as medicines is plant soursop (*A. muricata* L). Bioactive compounds found in soursop is annonaceous acetogenin(Setiadi, Z Zein, & Nauphar, 2019). Studies that prove the efficacy of acetogenins content in various countries financed by the Institute of the National Cancer Institute, USA provide results that stem *A. muricata* Linn. have cytotoxicity against cancer cells (George, Kumar, Suresh, & Kumar, 2015).

Soursop has been studied since the 1940s, phytochemical content that has been studied from this plant is acetogenins, alkaloids, quinolines, isoquinolines, tannins, Methanolic, coumarin, procyanidins, flavonoids, Acetaldehyde, Amyl-caproate. All parts of the soursop plant can be used for treatment (Sawant & Rajendra, 2014). Stems and leaves have annonaceous acetogenins substance that shows the cytotoxic active against cancer cells (Md Roduan, Hamid, Cheah, & Mohtarrudin, 2018). On the stem and soursop fruit had pesticide substances, but the leaves do not exist. Annonaceous acetogenins are only found in the Annonaceae family is a large group of phytochemical which naturally has anti-cancer activity (Arnab, Das D, & Mukherjee, 2017).

II. Research Method

II.1 Sample Preparation

Samples stem bark soursop (*Annona muricata* L.) from Kendari, Southeast Sulawesi. Stem bark powder soursop (*Annona muricata* L.) weighed as much as 550 grams inserted in a vessel maceration. Added solvent n-hexane as much as 1 L (Najib, Alam, & Halidin, 2012). Then silenced for 3x24 hours and occasionally stirring. Subsequently the filtrate was added with ethyl acetate solvent as 1L. Then let stand for 3x24 hours and occasionally stirring (Najib, Ahmad, & Handayani, 2019). Then the filtrate was added again with ethanol as much as 1 L, settling for 3x24 hours and occasionally stirring. Remaceration does as much as 1 L, settling for 3x24 hours and occasionally stirring. Then evaporated using a rotary evaporator and the liquid extract (Najib, Hartati, & Elya, 2011).

II.2 Sample Isolation

The results of profile TLC extract n-hexane by thin-layer chromatography using the eluent n-hexane: ethyl acetate (7: 3), the ethyl acetate extract using eluent n-hexane: ethyl acetate (7: 3), the ethanol extract using eluent n -hexane: ethyl acetate (6: 4) (Ahmad Najib et al., 2019). Then each plate sprayed with DPPH to test free radicals (Najib, Risda, Waris, Esti, Dian Pratiwi, 2016). Subsequently isolated by column chromatography using adsorbents silica gel 60 (0.2-0.5 mm) 60 grams of liquid elution with n-hexane: ethyl acetate (7: 3) (Syarif, Rasyid, & Najib, 2015). Then performed preparative thin layer chromatography using the eluent n-hexane: ethyl acetate (6: 1) (Najib, Alam, & Halidin, 2012).

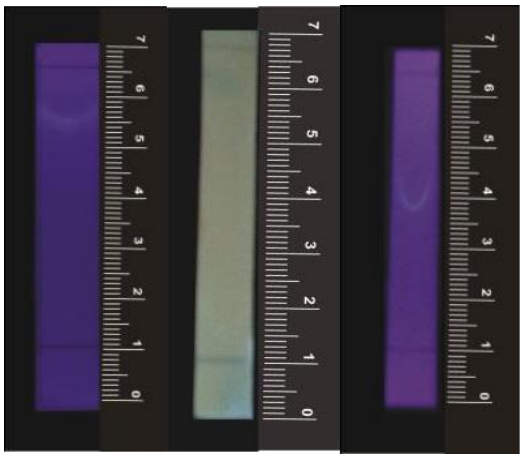
Test purity by TLC in two dimensions. Isolates obtained dissolved in methanol p.a, then spotted on a silica gel 60 F254 plates with a size of 5 x 5 cm. Then eluted with eluent acetone: ethyl acetate (10: 1) to the first direction, and chloroform: methanol (3: 3) for the elution process that is second done by rotating the plate counter-clockwise so that the results of the elution of the first to become the starting point elution for the second elution process (Hamidu, Ahmad, & Najib, 2018). If there is one single spot, it can be said that the isolate is a single chemical component, then isolates have been purely by TLC (Malik, Kurniawan, & Najib, 2014).

Purity test isolates were also performed using several variations of the eluent is chloroform: methanol (3: 3), acetone: ethyl acetate (10: 1) (Hartati, Elya, & Najib, 2010). Isolates were identified chemical that is sprayed with reagent $FeCl_3$, and $AlCl_3$ citroborat. The next analysis UV-Vis spectrophotometry measured the wavelength of 200nm - 250nm and Infrared spectroscopic analysis (Hamidu et al., 2018).

III. Result and Discussion

Samples stem bark soursop (*Annona muricata* L.) as much as 550 grams was extracted by maceration method using a graded solvent n-hexane, ethyl acetate and ethanol, to produce n-hexane extracts dried as much as 2.4 grams, the ethyl acetate extract dried 8.4. gram, ethanol extract dried as much as 2.1 grams (Hamidu et al., 2018).

The results of profile TLC showed free radical activity in the ethyl acetate extract stem bark soursop spot with Rf value of 0.6 which shows a color change from purple to yellow. Subsequently isolated by column chromatography resulted in 4 fractions. Subsequently the fraction 3 from the chromatography column showing free radical activity was isolated again by preparative thin layer chromatography using the eluent n-hexane: ethyl acetate (6: 1). Purity test isolates produce a single spot that can be viewed using UV 254 and UV 366 (Ahmad Najib et al., 2019). Identification by the chemical spraying $FeCl_3$, and $AlCl_3$ citroborat give negative results as shown on figure 1.



a **b** **c**

Figure 1. Specific Reagents for the class of phenolic and flavonoids compounds

In picture 1a using sitroborat reagents, picture 1b using $FeCl_3$ reagent and picture 1c using $AlCl_3$ reagent. Color identification Results showed negative results.

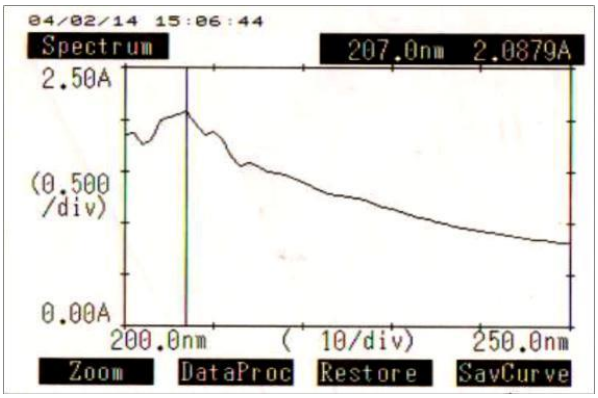


Figure 2. The results of UV-Vis spectrophotometry Isolates stem bark Soursop

Figure 2 showed the data of Spectroscopic Ultraviolet -Visible that resulting 3 isolates showed maximum absorption at a wavelength of 207 nm is measured from wavelength 200nm - 250nm. The compound that has a wavelength of 207 nm ± are aliphatic compounds (Nurul et al., 2016).

Infrared spectroscopy data on 3rd isolates as showed on figure 3, with spectrum data that is on the wave number 1093.80 cm^{-1} indicates the CO group in the area between 1000 cm^{-1} and 1300 cm-1 and the wave number 3327.68 cm^{-1} indicating OH group in the area of 2700 cm^{-1}, 3800 cm^{-1} (Silversten, Webster, Kiemle, & Bryce, 2015).

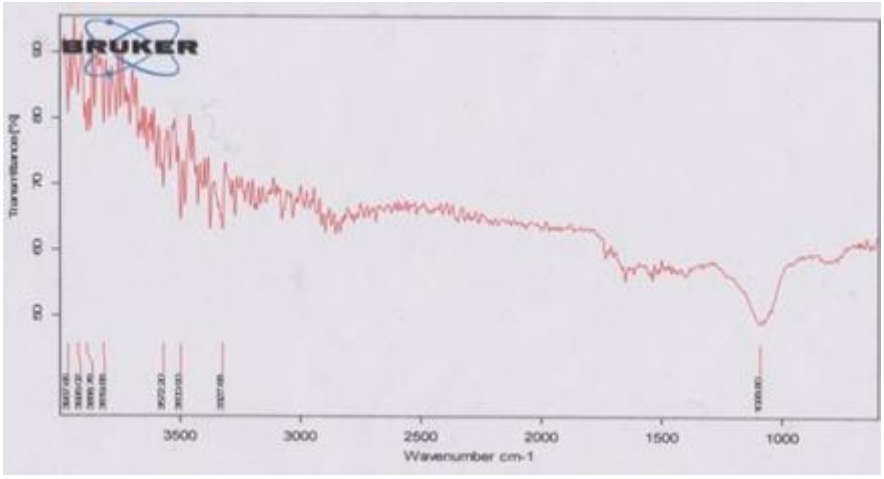


Figure 3. Spectrum Infrared isolates stem bark soursop

IV. Conclusion

From the results it can be concluded that the groups chemical compounds active free radical in isolates 3 stem bark soursop (*A. muricata* L.) is a class of acetogenin.

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