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Identification of acetogenin compound, sabadelin from soursop seeds, *Annona muricata* (Annonaceae)

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Abstract

Isolation and identification of one chemical compound from the seeds of soursop, *Annona muricata* (Annonaceae) based on bioactivity-guided fractionation by the Brine Shrimp Lethality Assay (BSLA) have been done. The powder of soursop seed was extracted successively with n-hexane, chloroform, and methanol then the methanol extract was partitioned with ethylacetate water. Isolation and purification of ethylacetate extracts were carried out by column chromatography [(SiO_2; *i. n*-hexane-chloroform = 15:1 ~ 1:1 gave one pure isolatedcompound. Based on Ultra Violet (UV), Infra-Red (IR), Nuclear Magnetic Resonance (NMR), and mass spectra (LC-MS/MS), the isolated Fr-4 was an acetogenin compound, sabadelin which had a weak cytotoxicity LC_{50} of 487 ppm.

Keywords: *Annona muricata;* Acetogenin; Sabadelin; Cytotoxic; Brine Shrimp Lethality Test

1. Introduction

As a continuation of previous research in the search for bioactive compounds from Indonesian medicinal plants, such as quercitrin from the leaves of *Dendrophthoe pentandra* (L.) Mig as antioxidant and antibacterial (Hadiyanti R. *et al*, 2019; Sembiring H. *et al*, 2022);anticancer from rodent tuber, *Typhonium* flagelliforme (Sianipar NF. *et al*, 2020); phenolic compounds from halban leaves, *Vitex pinnata* Linn as an antioxidant (Mastura *et al*, 2020); *n*-hexane extract of *Myrica fragrans* as an antioxidant and antitumor activities against MCF-7 cell line (Ginting B.*et al*, 2020); stilbinoids compound from the bark of Raru, *Vatica pauciflora* Baume has cytotoxicity (Kartika R. *et al*, 2021); and the neem plant, *Azadirachta indica* A. Juss has bioinsecticide activities (Prianto AH. *et al*, 2022). During this time we studied the chemical content of soursop seeds, *Annona muricata* (Fig. 1) as cytotoxic compounds. Annona is a genus of tropical fruit trees belonging to the family Annonaceae, of which there are approximately 119 species. Seven species and one hybrid are grown for domestic/commercial use.*Annona muricata* L. is known as soursop in English-speaking countries and is referred to by numerous common names. In Indonesia its called sirsak, nangka belanda, or nangka seberang (Coria-Telez AV. *et al*, 2018). The Annona species were distributed throughout the tropics. Soursop trees are widespread in the tropics and frost-free subtropics of the world and are found in the West Indies, North and South America, lowlands of Africa, Pacific islands, and Southeast Asia. The soursop fruit and other parts of the tree are considered to be underutilized. Information on the composition, nutritional value, medicinal uses, and toxicology of the soursop fruit and plant is limited and scattered (ICUC, 2008).

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Soursop fruit is quite large between 20-30 cm with a weight of up to 2.5 Kg.Soursop seeds are blackish-brown and hard, blunt-tipped with a shiny smooth surface with a length of approximately 16.8 mm and a width of 9.6 mm. The number of seeds in one fruit varies ranging from 20 – 70 seeds. Soursop seeds are a lot of waste that is discarded after the fruit is taken (such as in the fruit juice industry).

The nature of vegetable pesticides from soursop seeds occurs because soursop seeds contain extractive substances that act as antifeedants (anti-eating/blocking eating insects) and repellent (insect repellent) so that they can cause death in insects.

In general, seeds from plants contain carbohydrates in the form of starch (80%), cellulose (2-5%), and hemicellulose (7-15%). While Soursop seeds are rich in oil (22.10%) and protein (21.43%). Soursop seed oil consists of 28.07% saturated fatty acids and 71.93% unsaturated fatty acids consisting of 41-58% oleic acid, 12.33% linoleic acid, 16% palmitic acid, and 5% stearic acid).Annonaceous acetogenin compounds can inhibit cell growth and are toxic, antimalarial, antiparasitic, antimicrobial, antibacterial, and antifungal, reduce high blood pressure, normalize the nervous system that is not good, and as a natural pesticide(Badrie N. & A.G. Schauss, 2010).The active compound in the soursop plant is the annonaceous acetogenin compound which may have cytotoxic potential (compounds that can be toxic to inhibit and stop the growth of cancer cells) (Fertilita S *et al*, 2020). Annonaceous acetogenin compounds in soursop plants may be spread on all parts of the plant including soursop seeds, although with different concentrations. *Annona muricata* has many properties reported as antidiabetic, antitumor, antioxidant, antimicrobial, antibacterial, antimalarial-and others. However, studies on the use of fruit seeds are limited. In this study, we identified an acetogenin compound of *A. muricata* seed based on bioactivity-guided fractionation with shrimp larvae (*Artemia salina*).

A B C

Figure 1 Soursop tree, *Annona muricata* (A); seeds in the fruit (B); dried seeds (C)

2. Materials and methods

2.1. Plant material

The seeds of soursop (*Annona muricata*) collected from Pasar Bogor, West Java, Indonesia, and powdered with blended. All the chemicals and solvents were procured as an analytical grade. Thin Layer Chromatography (TLC) analysis used by TLC on pre-coated aluminum sheets, silica gel $GF_{254/366}$ (20 cm x 20 cm, 0,15 mm thickness, Merck) and compound were detected under UV light (254 & 366 nm) and spraying with $Ce_2(SO_4)/H_2SO_4$ with 5% methanol followed by heating the plates at 110°C for 5 minutes.

2.2. Extraction and Isolation

Soursop seeds (200 g) are blended, and dried. Then it was percolated with *n*-hexane, chloroform, and methanol successively.The methanol extract was partitioned into water and ethylacetate and then evaporated. Isolation and purification of ethylacetate extracts (5,0 g) were carried out by column chromatography [(SiO_2; *i*. *n*-hexane-chloroform = 15:1 ~ 1:1 giving eight fractions (Fr. EA-1 ~ Fr. EA-8), and then performed a cytotoxic assay against *Artemia salina*.Column chromatography was carried out on a silica gel column (70-230 mesh).

2.2.1. Brine Shrimp Lethality ASSAY (BSLA)

The cytotoxic activities of extracts and fractions were performed using Brine Shrimp Lethality Assay (BSLA) method described by Sarah QS*et al*. (2017)with slight modification

2.3. Spectroscopic Data Collection

Pure isolate (Fr. 4) and has the highest cytotoxicity to *Artemia salina* larvae, then measured in Ultra Violet (UV), Infra-Red (IR), Nuclear Magnetic Resonance (^{1}H &^{13}C NMR) spectra, and molecule weight (MW) measurement by LC-MS/MS. The Infra-Red (IR) spectra were recorded on the Prestige 21 FT IR (Shimadzu) model using KBr pellets. ^{1}H &^{13}C-NMR spectra of the compound in $CDCl_3$ have been recorded on Jeol 500 MHz NMR spectrophotometer. Mass spectral data of the prepared compound were measured on LC-MS/MS instruments. The absorbance measurements were carried out using a UV-Vis spectrophotometer (Shimadzu, model no: UV-1900) with 1 cm quartz cuvettes.

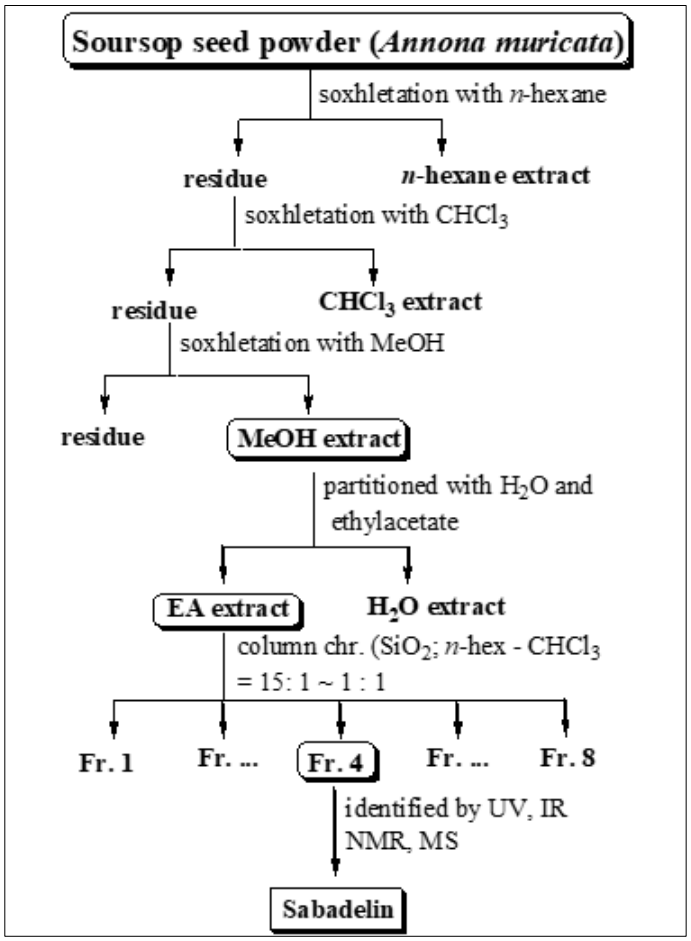

Figure 2 Scheme of Isolation and Purification of Sabadelin (1) from soursop seed (*Annona muricata*)

3. Results ad discussion

Soursop fruit is obtained from plantations in the Ciapus area, the fruit that has been obtained is then separated from the other parts so that only the seeds are obtained which are then washed and dried under direct sunlight and with the help of an oven. Soursop seeds that have been dried are then mashed using a milling machine. Simplisia dried soursop seeds are brown and have a distinctive smell. The water content of soursop seeds by following under the Indonesian herbal pharmacopeia, which is 6.17% obtained through a gravimetric process. Of the three extracts (*n*-hexane, chloroform, and methanol), the methanol extract had the best cytotoxic effect with an LC_{50} value of 104 ppm. The cytotoxic effect of the extract can be seen in Figure 3 (a).

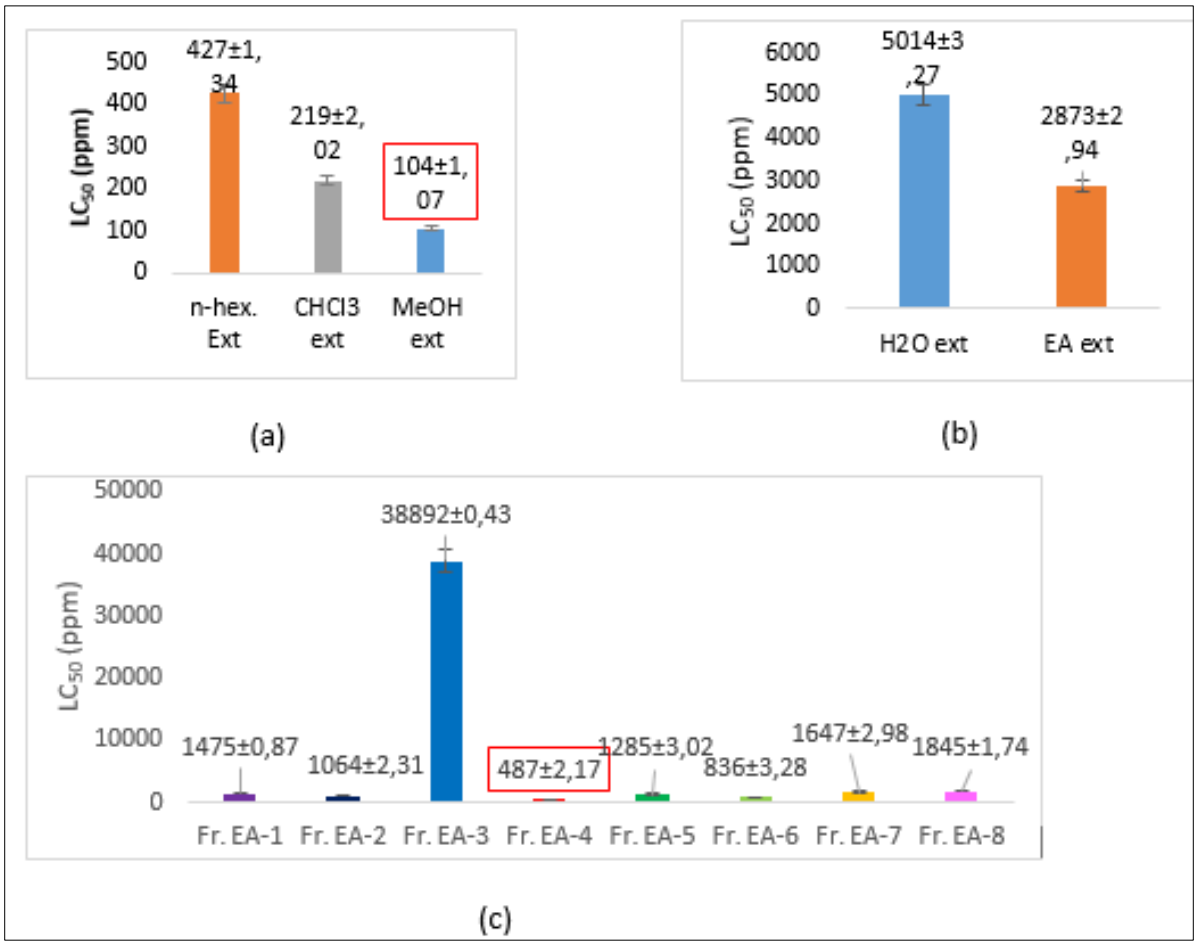

Figure 3 Cytotoxic effect of the extract (a), Partition (b), and Fractionation (c)

The methanol extract has a very strong cytotoxic effect, made be it's because methanol is a universal solvent, it can attract all active compounds, and each of these compounds synergizes with each other in a cytotoxic effect. A cytotoxic assay using *Artemia salina* is a preliminary test to predict the anticancer and pesticideproperties of an extractbecause the cells in *Artemia salina* have similarities to cancer cells. LC_{50} value was obtained to determine the amount of concentration that can kill the cell. then the smaller the LC_{50}, the more toxic it is to cancer cells and the better it is as an anticancer candidate (Andini *et al*., 2020)**.**

In the methanol extract, all of the compounds from non-polar to polar work to provide a cytotoxic effect on *Artemia salina*. To find out more specifically which compounds play a role, separation is carried out based on their polarity using the partition method and column chromatography. Partition results showed that the ethyl acetate fraction had a better effect than the water fraction. this shows that semi-polar compounds have a better role than polar compounds. the ethyl acetate fraction had a better cytotoxic effect than the water fraction with an LC_{50} of 2873 ppm. The cytotoxic effect of the fraction can be seen in Figure 3 (b).

Analysis of column chromatography gave 8 fractions and showed that fraction 4 was the best fraction with an LC_{50} of 487 ppm in the medium toxic category.(Clarkson C. *et al*, 2004). The LC_{50} value ofthe Brine Shrimp Lethality Assay well correlated with the IC_{50} value obtained by cytotoxic testing of soursop leaf extract against breast cancer cells through the *in vitro* MTT assay showed that the ethanol extract had a very strong IC_{50} and correlated well with the LC_{50} value (Corina F. *et al*, 2020). The results of previous studies revealed that the methanol extract produced from the partition of the ethanol extract using water, *n*-hexane, and methanol from soursop leaves had a toxicity of 2.8 ppm and *n*-hexane 83.8 ppm (Krisanti, *et al*, 2021). This difference is because *Annona muricata* uses slightly different leaves and partitioning methods.Bhanuwati conducted a similar study in 2022 by *in vitro* testing on HSC3 cancer cells using leaf extracts with various solvents, stating that the ethyl acetate extract had better activity than ethanol and *n*-hexane with an IC_{50} value of 76.66 ppm in the strong category. This was made possible because qualitatively the content of the ethyl acetate extract included alkaloids, flavonoids, and steroids. While the ethanol extract only contains saponins and

tannins, the *n*-hexane extract contains only triterpenoids and steroids (Bhanuwati, AV, *et al*, 2022). The results of the cytotoxic test of extracts and fractions can be seen in Figure 3(c).

The isolated compound (Fr. 4) is yellowish-white solid wax. The molecular formula of (**1**) was determined to be $C_{35}H_{62}O_3$ based on mass measurement of the LC-MS/MS *m/z* 531.2721 $[M+H]^+$; *m/z* 513 $[MH-H_2O]^+$. The ultraviolet (UV) spectra show the maximum wavelength at λ214 nm (in MeOH) and Infrared (FT-IR) gives the wavelength at atν2925; 1747; 1465;1375; 1110 cm^{-1} which indicates the presence of a carbonyl group (C=O), olefinic and epoxide group. In addition, the presence of an epoxide group is indicated by two protons resonance at δH 2.94 (m, H-17); δH H 2.90 (m, H-18), and δC 58.31 (C-17); δC 57.12 (C-18). The presence of a double bond was evidenced by multiple resonances due to two olefinic protons at δH 5.41 ~ 5.39 (H-13, H-14) and two carbon peaks at δC 128.11 (d, C-13) and δC 130.21 (d, C-14); and δH 6.16 (d, *J*=7.2 Hz; H-33) δC 149.12 (d, C-33); δC 130.01 (s, C-2). The configuration of the double bond was assigned as *cis* by comparing the chemical shift of ^{1}H &^{13}C-NMR data of isolated compound sabadelin by Gleye, C *et al*, 1999; Ragasa CY, *et al*, 2014). The comparison of the proton and carbon chemical shift (δH and δC) of Fr. 4 with the compound isolated from the roots of *Annona muricata* by Gleye (1999), therefore the chemical structure of fraction 4 can be determined as sabadelin (Table 1).

Table 1 The chemical shift (**δ**H &**δ**C) for isolated compound and sabadelin

No	**Sabadelin*) (δC, $CHCl_3$)**	**Isolated compound. (δC, $CHCl_3$)**	**Sabadelin*) (δH, $CHCl_3$)**	**Isolated compound. (δH, $CHCl_3$)**
1	173.9 (s)	172.82 (s)	-	-
2	134.3 (s)	130.01 (s)	-	-
3	25.2 (t)	25.08 (t)	2.26 (t)	2.76 (t, J=6,5)
4	27.2 (t)	27.21 (t)	1.55 (m)	1.59 (d, br
5~10	26.6-29.7 (t)	26.66~29.71 (t)	1.25~1.29 (m)	1.21~1.30 (m)
11	26.6-29.7	26.66~29.71 (t)	1.32 (m)	1.30 (br)
12	27.4 (t)	27,22 (t)	2.05 (m)	2.04 (m)
13	128.3 (d)	128,11 (d)	5.41 (m)	5.33 (m)
14	131.0 (d)	130,21 (d)	5.39 (m)	5.26 (m)
15	24.3 (t)	22.72 (t)	2.22 (m)	2.22 (m)
16	28.0 (t)	27.26 (t)	1.58 (m)	1.59 (br)
17	57.3 (d)	58.31 (d)	2.93 (m)	2.90 (m)
18	56.8 (d)	57.12 (d)	2.91 (m)	2.90 (m)
19	27.9 (t)	29.16 (t)	1.50 (m)	1.59 (br)
20~29	26.6-29.7 (t)	26.66~29.71 (t)	1.25~1.29 (m)	1.21~1.30 (m)
30	31.9 (t)	31.95 (t)	1.25~1.29 (m)	1.21~1.30 (m)
31	22.7 (t)	22.72 (t)	1.25~1.29 (m)	1,\.21- 1.30 (m)
32	14.1 (q)	15.00 (q)	0.88 (t)	0.88 (t)
33	148.3 (d)	149.12 (d)	6.98 (d)	6.16 (d, *J*=7.2)
34	77.4 (d)	75,98 (d)	4.99 (dq)	5.14 (d,*J* =7.2)
35	19.2 (q)	18.81 (q)	1.41 (d)	1.22 (d, *J* =7.0)

*): C. Gleye *et al*., 1999

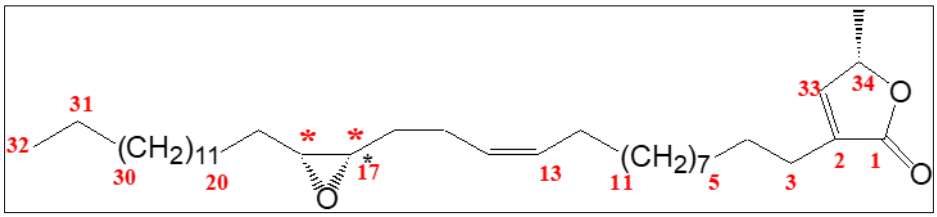

Figure 4 Chemical structure of fraction 4, sabadelin (* absolute configuration may be inverted)

Other acetogenin groups have been isolated from *mustur muricata* seed extract, including; muratenol; 2,4-cis-gigantetrocinone ; 2,4-transgigantetrocinone ; 2,4-trans-isoannonacin-10-one; 2,4-trans-isoaiinonacin; gigantetrosin-A; gigantetrosin-B ; 1-annomontasin and gigantetrocinin. Acetogenin compounds from the $CHCl_3$ fraction obtained through ethanol extract partitioned with air and $CHCl_3$ with strong antitumor activity (Li, DY, *et al*, 2001). Acetogenin is a class of polyketide compounds that are useful for the treatment of, among others, antibacterial, antiviral, antifungal, antiparasitic, antihypertensive, and antistress, and nourishes the system nerves. acetogenin use increasing especially in field health (ananti-cancer) and insecticides vegetables, derived from soursop leaves and seeds (HerreraNJ, 2019).

4. Conclusion

One acetogenin compound, sabadelin from the Indonesian medicinal plant the soursop seed, *Annona muricata* has been isolated and identified which has a weak cytotoxicity against *Artemia salina* of 487 ppm.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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