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Stigmasterol (Steroid) From Leaves of Solanum ferox L (Sour Eggplant) Plant

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Abstract: *Solanum ferox* L is a vegetable that is often used as a flavor enhancer in cooking that belongs to the Solanaceae family. *Solanum ferox* L contains terpenoids, steroids, flavonoids, alkaloids, and phenolic compounds. Solanum ferox has various bioactive compounds used as antirheumatic, antiasthma, antiviral, anticancer, and antibacterial. The steroid compound has been isolated from the leaves of *Solanum ferox* in n-hexane and separated using several chromatographies and it has been pure isolated the crystal colorless compound (0,0161 g). The chemical structure of these compounds is identified on the basis of spectroscopic data including IR, and 1D-NMR, along with a comparison with the spectral data previously reported. The comparison of experimental and reported NMR data, it known compounds were identified as Stigmasterol with the formula molecule $C_{29}H_{48}O$.

Keywords: *Solanum ferox; Solanaceae; Stigmasterol*

INTRODUCTION

Indonesia is a country that has abundant biodiversity. This makes Indonesia as one of the organic compound sources in the world. Indonesia's enormous biological wealth has the potential to be used as a source of medicine, natural insecticides, cosmetic ingredients, as well as natural flavoring and coloring for food. Plants contain complex and diverse chemical compounds (Abdullah et al., 2012). The contents of these compounds are grouped into primary metabolites and secondary metabolites (Newman & Cragg, 2007). Primary metabolite compounds are compounds resulting from the metabolism needed to support growth in every organism. Meanwhile, secondary metabolites are small molecules that are produced in a limited manner by organisms. In general, secondary metabolites have specific bioactivity and function as plant protectors against pests and diseases (Syarpin et al., 2018).

Solanum is a species from the Solanaceae family which is known to be rich in bioactive compounds (Olmstead et al., 2008). The sour eggplant plant is used by the Dayak community as a food flavoring (Cardenas et al., 2015; Hazimah, Teruna HY, 2013; Hazimah et al., 2019; Sumarlin et al., 2015). *Solanum ferox L* called as terung pasai (Brunei), terung asam dan cung bulu (Indonesia), terung dayak, terung iban dan terung asam (Malaysia), khua khon (Laos), tabanburo; tagatum (Filipina), sinkade (Myanmar), mapu; yongkuidi (Vietnam) dan muuk (Thailand) (Hazimah et al., 2018). Two

sesquiterpene compounds that are cytotoxic were isolated from *Solanum lyratum* and named solajiangxins F and G (Yadav et al., 2012). A flavonoid compound has been isolated from *Solanum verbascifolium* named 7,4′-dimethyl-apigenin-6-C-β-glucopyranosyl-2″-O-α-l-arabinopyranoside (Ohtsuki et al., 2010). Two alkaloid compounds that have anti-breast cancer properties from *Curcuma xanthorrhiza* are named erianosides A and B (Atun et al., 2022).

Solanum ferox is widely used by the community to treat various diseases, that is syphilis, body aches, lack of appetite, fever, itching, wounds, bruises and antipyretics. *Solanum ferox* is reported to have pharmacological activity, such as antioxidant and antibacterial (Hazimah, Teruna HY, 2013). Active compounds from plants can be extracted with various organic solvents, such as hexane, ethyl acetate and methanol, various active secondary metabolites have been isolated from the n-hexane fraction (Syarpin et al., 2018) and n-Hexane is a decent solvent when applied to extract non-polar compounds because it has various advantages, namely being volatile, stable, and selective. In this study, the isolation and characterization of the n-hexane fraction of stigmasterol compounds from the leaves of *Solanum ferox* was carried out.

RESEARCH METHODS

Tools and Materials

The tools used are a rotary evaporator unit, maserator, mortar, distillation equipment, chromatography column, TLC plate, ultraviolet lamp (Camag®) 254/366 nm, Fisher John Melting Point Apparatus (SMP 11-Stuart®), analytical balance, UV spectrophotometer-Vis 10S Genesis, IR spectrophotometer Shimadzu brand Prestige 21 type, NMR spectrophotometer brand JOEL type ECA 500 with a frequency of 500 Hz for protons and 125 Hz for carbon, vials, aluminum voil, and glassware commonly used in laboratories. The materials used were n-hexane, ethyl acetate, methanol, silica gel 70-230 mesh, silica gel 60 GF254, silica gel TLC plate GF254, solvents for spectroscopic analysis (UV, IR and NMR) were CDCl3, cotton, aluminum foil and aquades.

Procedures

Sampel Preparation

The sample used in this study was the leaves of *Solanum ferox* plant which was planted at COMPOS FMIPA, University of Riau. *Solanum ferox* plants which had been dried in the oven at 40^0C were cut into small pieces until smooth. Then weighed several times until the weight is constant.

Isolation of chemical compounds from Solanum ferox L

The dried samples from leaves of *Solanum ferox* were macerated and ultrasonicated several times using n-hexane solvent until colorless. The colorless solvent was collected and the solvent was evaporated using a rotary evaporator to obtain a thick n-hexane extract.

Separation by VLC (Liquid Vacuum Chromatography)

Extract was fractionated using liquid vacuum chromatography to separate the compounds in the n-hexane extract. Liquid vacuum chromatography (3 cm in diameter and 20 cm in height) was filled with silica gel $_{60}$ GF_{254} to a height of approx 5 cm. The Column filling is carried out in a vacuum, in order to obtain maximum packing density. The n-hexane extract to be fractionated was pre-adsorbed and put into the column. Then

eluted in a gradient manner using n-hexane solvent (100 ml), the ratio of n-hexane-ethyl acetate (60 ml:40 ml). The results of the separation were accommodated in a numbered erlemeyer and then a TLC test was carried out to determine the number of components of each fraction. Fractions 1, 2 and 3 of the hexane extract gradient were combined because they had the same spot pattern, so there were 6 fractions.

Separation by column chromatography

1.25 grams extract of n-hexane was separated using column chromatography. The column can be prepared by making silica gel slurry which functions as an absorbent by dissolving it in n-hexane and stirring thoroughly. Then it is poured into the column slowly. Silica gel in the column is made solid and the surface should not dry. Column elution was carried out with various eluents that had increasing polarity, for F3 from the VLC extract n-hexane results starting from eluent n-hexane (100%), n-hexane: ethyl acetate (9:1), n-hexane: ethyl acetate (8:2), n-hexane: ethyl acetate (7:3), n-hexane: ethyl acetate (6:4), so on until it reaches ethyl acetate: methanol (1:1) with an increase of 10%. The sample is pre-absorbed before being inserted into the column, then eluted with solvent to form bands. The fractionation results that come out are accommodated in vials that have been numbered. The fractions that were separated by column chromatography which revealed the presence of crystals were subjected to a TLC test to determine the same Rf (Retardation Factor) value so that they could be combined. The crystals formed were recrystallized with a solvent that does not dissolve in the cold state and dissolves in the hot state, then the recrystallized crystals were subjected to the TLC test.

Testing the results of separation with TLC

The fractions resulting from column chromatography separation were subjected to the TLC test by dabbing them on the TLC plate using a capillary tube. Each fraction was spotted on a numbered plate according to the vial number, then eluted with crude extract TLC results with a good stain separation pattern. The elution process in TLC, the eluent is put into the chamber so that the pressure is homogeneous, after the TLC homogeneous pressure is eluted to the upper limit of the plate, then the plate is removed and dried. Stains can be seen with an ultraviolet (UV) lamp or with a stain suppression reagent. The fractions having the same Rf value are combined and the solvent is evaporated.

Characterization

Five (5) mg of crystals were characterized using NMR, 0.2 mg crystals were characterized using UV spectroscopy at the UR FMIPA Biochemistry Laboratory, 0.1 mg crystals were characterized using IR spectroscopy at the IR Laboratory, UR FMIPA.

RESULT AND DISCUSSION

Solanum ferox leaves extract

500 grams of sour eggplant (*Solanum ferox*) leaves were extracted by maceration method using n-hexane solvent 3 repetitions for 24 hours and ultrasonication was carried out for 30 minutes to produce 14.29 grams of dark green extract, then the extract was subjected to a TLC test with comparison of Hexane and Ethyl acetate solvents (9:1) to check of components present in n-hexane viscous extracts.

Recrystallization and determination of melting point

Recrystallization was carried out using hot n-hexane solvent and white needle crystals weighing 0.0161 g were obtained. The crystals melting point was measured using the Fisher Johns tool. The melting point of the crystal is 125-126 ºC with standard melting point of stigmasterol is 127-128 ºC(Nurmalasari et al., 2016).

Characterization

White crystals were characterized using UV spectroscopy at a wavelength of 200 – 400 nm. UV spectroscopic analysis resulted in maximum absorption at a wavelength of 271 nm.

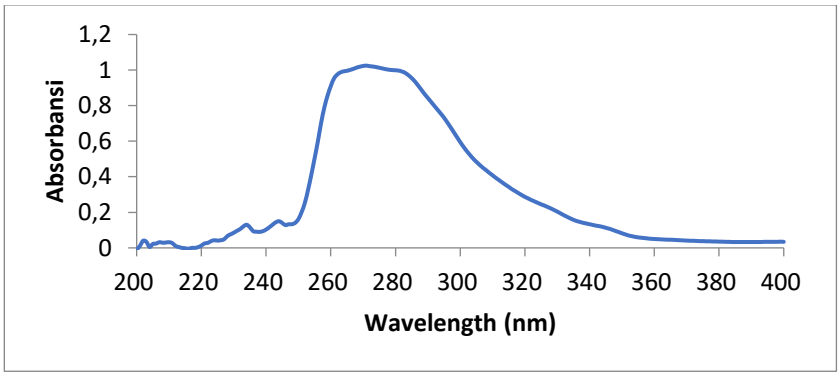

Figure 1. UV Chromatogram

This reveals that compound has no conjugated bonds, this is due to the presence of a carbonyl group, while the double bond is not clear due to overlapping spectra. This is supported by IR data which reveals absorption at wave number and 1666 cm^{-1} (compound) which revelas the presence of a C=C double bond.

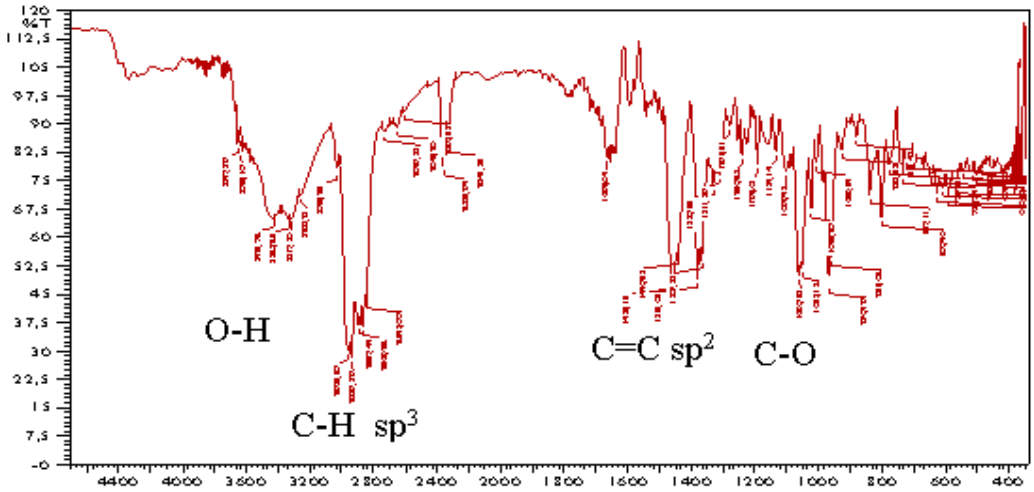

Figure 2. FT-IR Chromatogram

The IR spectrum (KBr) of the compound (Figure 2) reveals the presence of hydroxyl group (3421 cm-1), C-H sp3 (2958 cm-1), olefinic group C=C sp2 (1666 cm-1) and C-O group (1052 cm-1). The ^{1}H-NMR spectrum of the compound (Figure 3) reveals the presence of six sp3 methyl signals consisting of two tertiary methyl signals that resonate at δ_H 0.67 (3H, s) and 1.00 (3H, s), three secondary methyls at δ_H 0.92 (3H, d, J = 5.9 Hz), 0.84 (3H, d, J = 6.4 Hz), and 0.82 (3H, d, J = 6.4 Hz), one primary methyl signal that resonates at δ_H 0.80 (3H, t, J = 6.0 Hz) suggesting characteristics of steroid group compounds (Yayli & Baltaci, 1996). Three olefinic methine signals resonating at δ_H 5.35 (1H, d, J = 5.1 Hz, H-6), 5.18 (1H, dd, J = 8.5; 15.1 Hz, H-22), and 5.00 (1H, dd, J = 8.6; 15.6 Hz, H-23), as well as one oxygenated methin signal at δ_H 3.52 (1H, m, H-3) suggest characteristics of 3-β stigmasterol compounds (Cayme & Ragasa, 2004; Yayli & Baltaci, 1996).

The ^{13}C-NMR spectrum of the compound (Figure 3) reveals the presence of 29 carbon signals consisting of six methyl, ten methylene, eleven methine and two quaternary carbon signals that suggest steroid class compounds (Yayli & Baltaci, 1996). The presence of six methyl signals resonating at δc 12.40 (C-18), 19.56 (C-19), 21.24 (C-21), 21.30 (C-26), 19.14 (C-27), and 12.20 (C-29), one oxymetin signal at δc 71.98 (C-3), three methine olefinics at δc 121.87 (C-6), 138.46 (C-22), 129.43 (C-23), as well as one singlet olefinic carbon signal at δc 140.91 (C-5) suggest characteristics of stigmasterol compounds (Cayme & Ragasa, 2004; Yayli & Baltaci, 1996). Such functionality is calculated as two of the total six degrees of unsaturation. The remaining four degrees of unsaturation, correspond to the basic framework of steroid stigmastan (Cayme & Ragasa, 2004). Comparison of compound spectroscopic data with stigmasterol compounds (Cayme & Ragasa, 2004), it reveals that the two compounds have a very high degree of compatibility, thus the compounds are identified as stigmasterol (Figure 5).

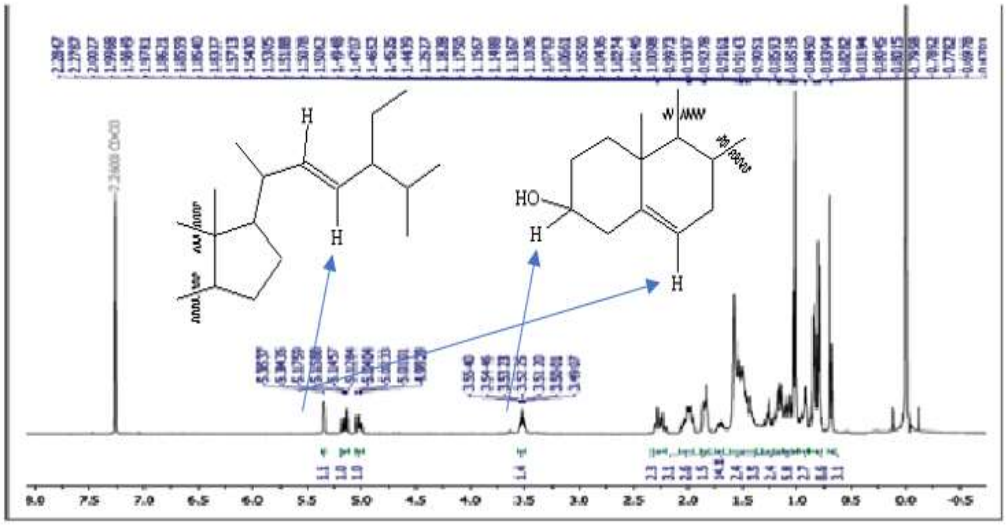

Figure 3. ^{1}H-NMR Spectrum

Ratio of the spectroscopic data of the compound and the compound stigmasterol (Katja, 2021) reveals that the two compounds have a very high degree of concordance, thus the compound is identified as stigmasterol (**Fig 5**).

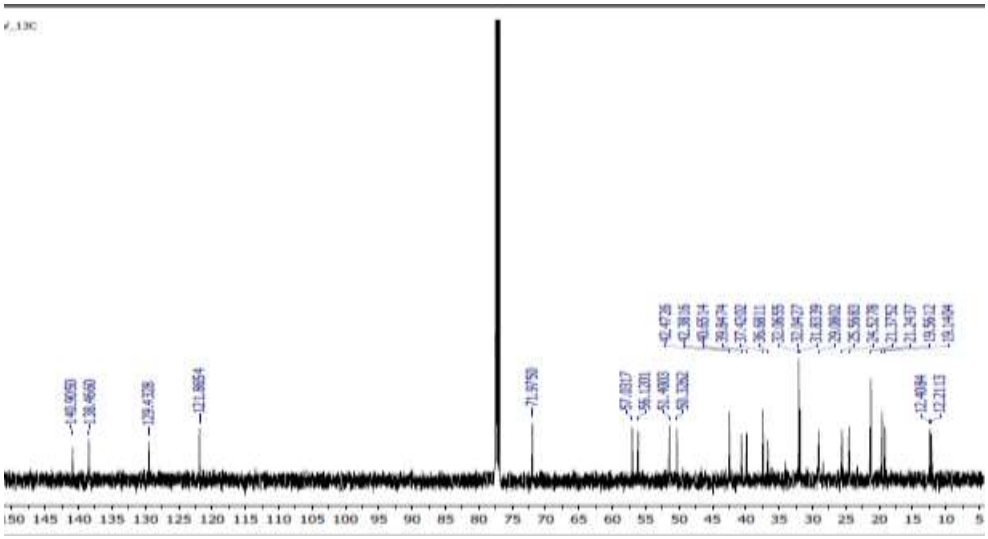

Fig 4. ^{13}C-NMR Spectrum

Comparison of compound spectroscopic data with stigmasterol compounds (Cayme & Ragasa, 2004), sreveals that the two compounds have a very high degree of compatibility, thus the compounds are identified as stigmasterol (Figure 5).

Tabel 1. Ratio NMR data with Stigmasterol (Katja, 2021).

No	Crystal		Stigmasterol	
	^{13}C NMR δc (mult)	^{1}H NMR δH (Integral, mult, J=Hz)	^{13}C NMR δc (mult)	^{1}H NMR δH (Integral, mult, J=Hz)
1	37.40	1.08 (1H, m)	37.4	1.08 (1H, m)
		1.84 (1H, m)		1.84 (1H, m)
2	31.80	1.49 (1H, m)	31.8	1.49 (1H, m)
		1.81 (1H, m)		1.81 (1H, m)
3	71.98	3.52 (1H, m)	72.0	3.52 (1H, m)
4	42.47	2.28 (1H, dd, 2.0;5.2)	42.5	2.28 (1H, dd, 2.0;5.2)
				2.30 (1H, dd, 2.0;5.2)
5	140.91	-	140.9	-
6	121.87	5.35 (1H, d, 5.1)	121.9	5.35 (1H, d, 5,1)
7	32.06	1.54 (1H, m)	32.1	1.54 (1H, m)
		1.96 (1H, m)		1.96 (1H, m)
8	32.04	1.46(1H, m)	32.0	1.46(1H, m)
9	50.33	0.94 (1H, m)	50.3	0.94 (1H, m)
10	36.68	-	36,.7	-
11	21.37	1.46 (1H, m)	21.3	1.46 (1H, m)
		1.49 (1H, m)		1.49 (1H, m)
12	39.85	1.15 (1H, m)	39.9	1.15 (1H, m)
		1.95 (1H, m)		1.95 (1H, m)
13	42.38	-	42.5	-
14	57.03	1.03 (1H, m)	56.9	1.03 (1H, m)
15	24.53	1.07 (1H, m)	24.5	1.07 (1H, m)
		1.56 (1H, m)		1.56 (1H, m)

16	29.08	1.26 (1H, m) 1.67 (1H, m)	28.4	1.26 (1H, m) 1.67 (1H, m)
17	56.12	1.13 (1H, m)	56.1	1.13 (1H, m)
18	12.40	0.67 (3H, s)	12.1	0.67 (3H, s)
19	19.56	1.00 (3H, s)	19.5	1.00 (3H, s)
20	40.65	2.02 (1H, m)	40.7	2.02 (1H, m)
21	21.24	0.92 (3H, d, 5.9)	21.2	0.92 (3H, d, 5,9)
22	138.46	5.18 (1H, dd 8.5;15.1	138.5	5.18 (1H, 8,5;15,1
23	129.43	5.00 (1H, dd, 8,6; 15.6)	129.5	5.00 (1H, dd, 8.6; 15.6)
24	51.40	1.53 (1H, m)	51.4	1.53 (1H, m)
25	31.80	1.45 (1H, m)	31.8	1.45 (1H, m)
26	21.30	0.84 (3H, d, 6,4)	21.3	0.84 (3H, d, 6.4)
27	19.14	0.82 (3H, d, 6,4)	19.1	0.82 (3H, d, 6.4)
28	25.57	1.51 (1H, t, 3,2)	25.6	1.51 (1H, t, 3.2)
29	12.20	0.80 (3H, t, 6,0)	12.2	0.80 (3H, t, 6.0)

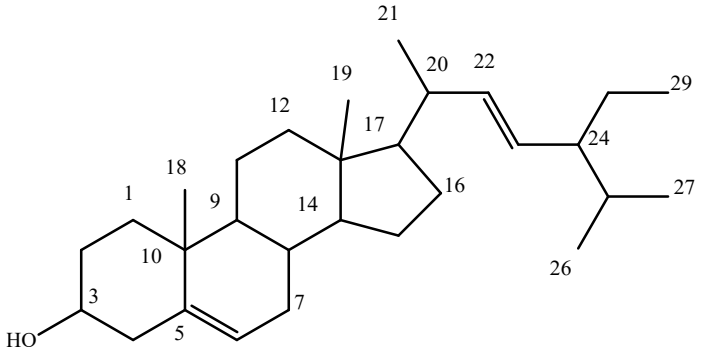

Figure 5. Stigmasterol

CONCLUSION

Based on the interpretation of the IR spectra, ^{1}H-NMR, ^{13}C-NMR and comparative data, the isolate was a colorless crystalline solid of 0.0161 g isolated from 500 g of dry powder of *S. ferox* leaves is stigmasterol.

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