11,12-dehidro-β-sitosterol Compound from the stem of Belajang Susu (Scindapsus pictus Hassk)

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Abstract

The belajang susu plant (Scindapsus pictus Hassk.) Is a species of the genus Scindapsus and belongs to the family Araceae which is used by the traditional Mambi community as an anticancer. Plants belonging to this genus are generally used as ornamental plants, but some are used as medicinal plants because they contain active compounds and are anti-inflammatory, analgesic, antioxidant and antitumor. Plants of S. pictus Hassk. reportedly contains oxalate which is usually in the form of calcium oxalate, flavonoids sulfate and a mixture of flavones and flavonol sulfates. Research methods include extraction, fractionation and identification using FT-IR and NMR spectrophotometers. The results obtained are compounds 11,12-dehydro-β-sitosterol in the form of needle crystals with a melting point of 132-134oC.

Keywords: 11,12-dehidro-β-sitosterol, Belajang Susu and S. pictus Hassk

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Introduction

Indonesia is one of the countries rich in biodiversity. In the world there are approximately 250.000 species of tall plants, and more than 60% of these are tropical plants (Atun, 2014). As many as 20.000 types of medicinal plants are found in Indonesia, but only as many as 1.000 species of plants are recorded and only 300 are used as traditional medicines (Wijaya et al., 2014).

Medicinal plants produce secondary metabolites that have the potential as bioactive compounds and are useful for human life. Each plant produces one or more bioactive compounds in the form of secondary metabolites, such as alkaloids, flavonoids, phenylpropanoids, steroids, terpenoids, tannins, and coumarin whose existence is highly dependent on the type of plant. This is what causes plants to be used as medicines for hundreds or even thousands of years ago.

One family of plants that are used as medicinal plants is Araceae. The main characteristic of this family is the flowering which is arranged in the form of cob (spadix) surrounded by a sheath (spathe) (Asih et al., 2015). This family includes 110 genera and 1800 species, spread in tropical

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and subtropical regions, approximately 23 genera are native to Malaya (Syamsiah, 2016). Species belonging to this family are rich in anthocyanin content in inflorescences, fruit, leaves or petioles (Kaur & Rajiv, 2016). In addition, this plant is generally known to be rich in active and structurally unique compounds, such as alkaloids, phenols, saponins, sterols and so on (Dong et al., 2018). Some species that can be used as medicinal plants such as Alocasia macrorrhiza Schott are used as cough medicines, Acorus calamus L is used as a sedative and Pistia stratiotes L is used as a whooping cough medicine, fever and for facilitating urine (Hutapea, 2000).

The belajang susu plant (S. pictus Hassk.) Is one of the species belonging to the family Araceae and the genus Scindapsus. Generally members of this clan are used as ornamental plants. However, some are used as medicinal plants (Yuzammi & Reza, 2015). Like Scindapsus officinalis whose methanol extract contains active compounds which are anti-inflammatory, analgesic, antioxidant and antitumor (Kaur & Rajiv, 2016). Based on the results of special research on local plant exploration in Indonesia based on the Mambi ethnic community from Mambi Subdistrict, Mamasa Regency, West Sulawesi, the belajang susu (S.pictus Hassk.) Plant is used as an anticancer (Jumadi et al., 2012). In addition, this plant is reported to contain oxalate which is usually in the form of calcium oxalate, flavonoid sulfate and a mixture of flavones and flavonol sulfates (Kaur & Rajiv, 2016).

Based on the chemotaxonomic and ethnobotany approaches, there is the potential to find secondary metabolite compounds in belajang susu plants (S. pictus Hassk.) Chemotaxonomic approach, namely the selection of plants based on the closeness of plants that are known to have certain chemical contents. Plants or other organisms in a family are often found producing naturally similar compounds. While the ethnobotany approach is the initial exploration of active ingredients of a plant based on the knowledge and habits of traditional communities in utilizing plants for the treatment of certain diseases (Mamahit, 2009). Therefore, the researcher considers that a research is needed to examine deeper the content of secondary metabolite compounds from methanol extracts of bare belajang susu stems (S. pictus Hassk.).

Method

The melting point of the compound was measured using the Melting Point Apparatus and the compound functional group was identified using the SHIMADZU® Prestige-21 FT-IR spectroscopy. The ^{1}H-NMR and ^{13}C-NMR spectra were obtained from the Agilent spectrophotometer DD2 console system.

The belajang susu is cleaned and cut into small pieces and then dried by aerating at room temperature to dry. After that, it is ground up to obtain 2,63 kg of milk banana powder, which is then macerated with methanol for 3 x 24 hours. The maserate is then filtered with a Buchner funnel and then concentrated using an evaporator to approximately a quarter of the initial volume. Furthermore, a preliminary test (group test) was carried out on methanol extracts obtained with various reagents including Liebermann-Burchard (steroid), $FeCl_3$1% (flavonoid), Dragendorff (alkaloid) and Wagner (alkaloid) reagents. The methanol extract obtained first was identified using thin layer chromatography (TLC). A small amount of the extract is bottled on the TLC plate using various eluents at various comparisons to find out the type of solvent and the corresponding ratio on the vacuum liquid chromatography column. Then detected under a UV lamp and followed by

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spraying a solution of cerium sulfate and then heated. Based on TLC results, it was found that the n-hexane: ethyl acetate (8: 2) eluent provided a good and clear separation pattern for vacuum liquid chromatography.

The first fractionation process was carried out by a vacuum liquid chromatography method, with 33,70 g of methanol extract of milk single stem in the form of paste impregnated with silica gel 60 (0.2-0.5 mm) until it was mixed evenly and in the form of sand. Then into the column placed successively from the bottom to the top of 60 GF_{254} silica gel at the very bottom (stationary phase) and pressed until solid, whatman filter paper, samples that have been impregnated. The packed column is then eluted with the appropriate solution as the mobile phase. The tracing starts from a nonpolar solvent that is 100% n-hexane then the polarity is increased by adding a mixture of n-hexane: ethyl acetate solvent starting at a ratio of 95: 5 and increasing in a gradient to 100% ethyl acetate and then eluent ethyl acetate-acetone in the ratio (95: 5) to 100% acetone eluent and finally with a polar solvent, 100% methanol. Faction obtained as many as 37 fractions were identified using TLC with the appropriate eluent. The fractions which have the same stain profile are combined so that 8 main fractions are obtained. The KKCV results obtained were evaporated at room temperature.

Fraction C weighing 1,74 g was selected for fractionation using the compressed column chromatography method. Before being fractionated it is first identified by TLC to determine the eluent to be used in the KKT. The column for fractionation with KKT is filled with silica gel 60 (0.063-0.200mm) up to ± 15 cm high as a stationary phase then filled with impregnated samples and the appropriate eluent as the mobile phase. The fractions obtained as many as 13 fractions were analyzed using TLC with the appropriate eluent. The fractions which have the same stain profile are combined and obtained 7 main factions then evaporated at room temperature. C_3 isolates obtained from the combined KKT fraction were purified with suitable solvents. The purity of the compound obtained was determined by performing a TLC of three eluent systems. If the TLC results show a single stain pattern, then the compound is relatively pure by TLC. Then the melting point test is performed and identified by FT-IR and NMR spectrophotometers.

Results and Discussion

11,12-dehydro-β-sitosterol compounds in the form of needle crystals with melting point 132-134^{o}C. Figure 1 shows the FT-IR spectrum of the compound.

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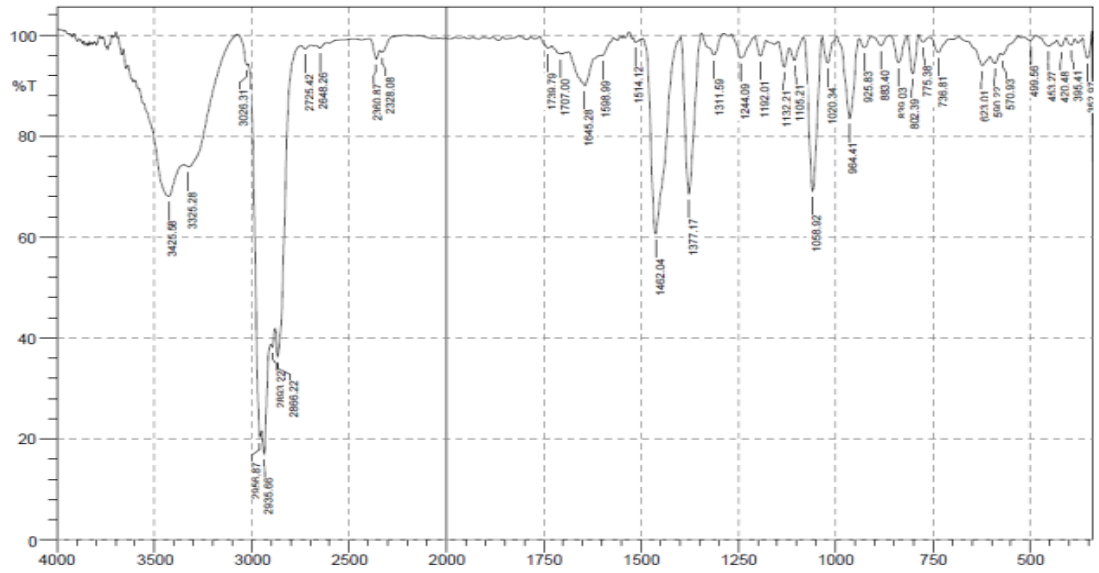

Figure 1. FT-IR Spectrum of11,12-dehidro-β-sitosterol

In the region of wave number (ν) 3425.58 cm^{-1} marked with a rather wide band with moderate intensity identified as O-H stretching vibrations. Then the presence of sharp absorption with moderate intensity in the region of wave number 1058.92 cm-1 was identified as C-O buckling vibrations. Sharp absorption with strong intensity was seen in the area of 2956.87cm^{-1}; 2935,66cm^{-1}; 2893.22 cm^{-1} and 2866.22 cm^{-1} which are the stretching vibrations of C-H aliphatic groups -CH_2 and -CH_3. This characteristic of aliphatic C-H is characterized by absorption in the 2800-3000 region. This indicates that the structure of isolate compounds contained methyl and methylene groups. The presence of methyl and methylene groups is strengthened by the presence of aliphatic CH buckling vibrations in the region of 1462.04 cm-1 for the -CH_2 group and 1377.17cm-1 for the -CH_3 group which indicates the presence of dimethyl-$CH(CH_3)_2$ as a characteristic compound of the CH_2 compound triterpenoids/steroids. Absorption by dimethyl geminal usually breaks into two peaks with the same intensity, but these two peaks are not always visible in all spectra, which is common in only one peak [11]. Sharp absorption with a weak intensity in the area of 1645.28 cm^{-1} indicates a stretching vibration of C = C (1600-1700 cm^{-1}) and is strengthened by an absorption at 964.41 cm-1 which is a bending vibration of = CH (800 - 1000 cm^{-1}).

The ^{1}H-NMR spectrum shows several typical signals, including two proton signals at δ 5.31 ppm (H-6) as a double bond proton and δ 3.38 ppm (H-3) as a proton that is influenced by the effect of oxygen induction with signal support 3-OH at 73 4.73 ppm (brs), as well as two methyl proton signals with singlet multiplicity at δ 0.74 ppm (H-18) and δ 0.82 ppm (H-19). These signals show the main characteristics of the steroid core framework, which is substituted for 1 hydroxyl group and 2 methyl groups. Furthermore, there are two proton signals in double bonds at 21 5.21 ppm (H-11, 1H, dd, J = 8.7 & 8.75 Hz) and δ 5.08 ppm (H-12, 1H, d, j = 8.7 Hz) which indicates the addition of a double bond in the steroid nucleus.

An additional framework of the steroid nuclei is also shown by ten aliphatic proton signals. These signals form an alkyl framework consisting of, 3 multiplet meth signals at δ 1.45; 0.97 and 1.55

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ppm (H-20; H-24 and H-25), 3 multiplet methylene signals at δ 1.74; 1.48 and 1.31 ppm (H-22; H-23 and H-28), and 4 methyl signals at δ0.81 ppm (H-21, d, j = 5.4 Hz); 0.88 ppm (H-26, d, j = 2 Hz); 0.85 ppm (H-27, d, j = 2.9 Hz) and 0.79 ppm (H-29, m).

The ^{13}C-NMR spectrum shows 29 signals representing 29 amounts of carbon, including one oxy carbon at δ 71.72 ppm (C-3), 4 carbon double bonds at δ 142.36; 121.55; 130.11 and 139.36 ppm (C-5; C-6; C-11: and C-12), and 24 aliphatic carbon signals. The DEPT-135 spectrum shows positive carbon signals consisting of 6 methyl carbons at δ 12.44; 19,37; 19.24; 19.82; 20.10 and 12.60 ppm (C-18; C-19; C-21; C-26; C-27 and C-29) and 11 carbon methines at δ 71.72; 121.55; 32.71; 51.23; 130.11; 139.36; 57.68; 56.84; 36.95; 46.74 and 32.55 ppm, as well as negative signals from 9 methylene carbon at δ38.26; 32.49; 43.11; 32.65; 24.96; 28.97; 34.72; 26.10 and 23.77 ppm. Based on the DPT spectrum, it is known that there are 3 quaternary carbon at yaitu 142.36; 37.34 and 42.99 ppm (C-5; C-10 and C-13). 29 carbon signals form a steroid skeleton with 2 double bonds at C-5 & C-6 and C-11 & C-12, and the C-17 position binds to the alkyl unit.

The ^{13}C-NMR spectrum data is significant with signal peaks shown in the β-sitosterol compound that have been reported by Ahmed (2013) / pince with a difference in signals for C-11 and C-12 by the presence of 2 hydrogen deficiency forming an additional 1 double bond two.

Based on the ^{1}H-NMR and ^{13}C-NMR spectrum data that have been described, it can be suggested the molecular structure of this compound with the name 11,12-dehydro-β-sitosterol, or with the name IUPAC; 17- (5-ethyl-6-methylheptane-2-il) -10,13-dimethyl-1,2,4,7,8,9,14,15,16,17-decahydro-cyclopentane [o] fenantren- 3-ol.

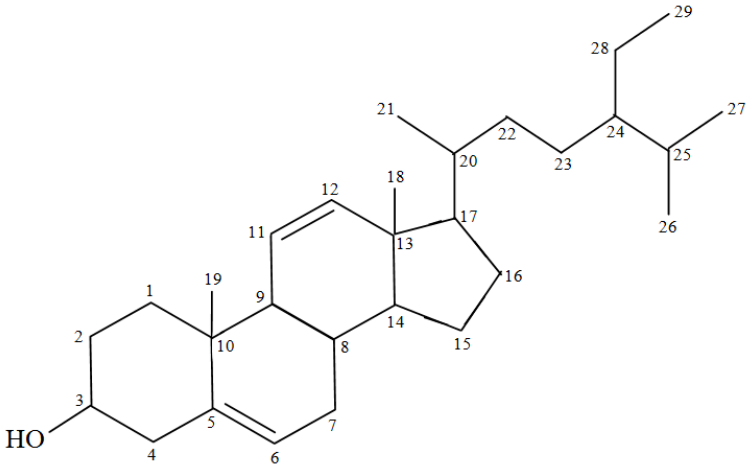

Figure 2. Compound Structure 17-(5-etil-6-metilheptan-2-il)-10,13-dimetil-1,2,4,7,8,9,14,15,16,17-dekahidro-siklopentan[o]fenantren-3-ol, atau senyawa 11,12-dehidro-β-sitosterol

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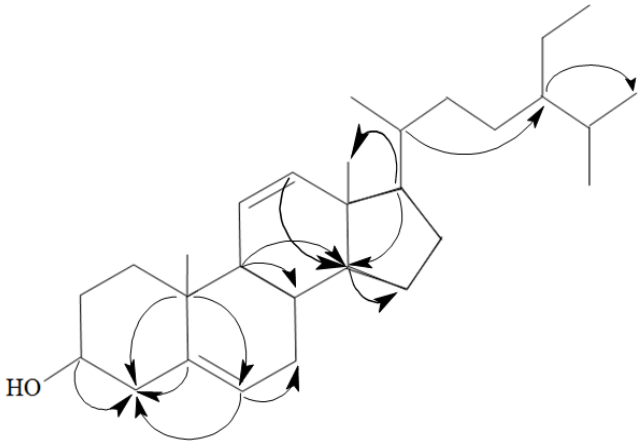

Figure 3. Correlation HMBC $^{13}C \rightarrow {}^{1}H$

The molecular structure of this compound is strengthened by the HMBC spectrum which shows the long distance correlation of carbon signals to proton signals, including signals at δ 71.72 ppm (C-3) to δ 2.18 ppm (H-4), δ 121.55 ppm (C-6) against δ 2.18 ppm (H-4) and δ 1.94 ppm (H-7), δ 139.36 ppm (C-12) against δ 1.06 ppm (H-14), and signals at δ 56.84 ppm (C-17) against δ 0.74 ppm (H-18). Complete data can be seen in table 1.

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Table 1. ^{1}H dan ^{13}C Spectrum of 11,12-dehidro-β-sitosterol compound

13C-NMR δ ppm	**1H-NMR δ ppm (H, multiplisitas, konst.kopling)**	**HMBC C→H**	**β-sitosterol**	
			Pince	**Ahmed (2013)**
38,26	1,85 (2H, m)	H-4	37,4	37,29
32,49	1,77 (2H, m)		31,8	31,95
71,72	3,38 (1H, m) 4,73 (3-OH, brs)		71,9	71,84
43,11	2,18 (2H, m)			
142,36	-	H-4	42,4	42,36
121,55	5,31 (1H, C, J=5,35 Hz)	H-4, H-7	140,9	140,80
32,65	1,94 (2H, m)		121,9	121,73
32,71	1,98 (1H, m)		32,09	31,71
51,23	0,95 (1H, m)	H-8, H-14	32,06	31,95
37,34	-	H-4, H-6	50,3	50,19
130,11	5,21 (1H, dd, J=8,7 & 8,75Hz)	H-14	36,6	36,18
139,36	5,08 (1H, d, J= 8,7 Hz)		21,2	21,12
42,99	-		39,9	39,82
57,68	1,06 (1H, m)	H-15	42,4	42,36
24,96	1,11 (2H, m)		56,9	56,81
28,97	1,25 (2H, m)		24,4	24,33
56,84	1,22 (1H, m)	H-14, H-18	28,4	28,26
12,44	0,74 (3H, s)		56,2	56,11
19,37	0,82 (3H, s)		12,0	11,88
36,95	1,45 (1H, m)	H-24	19,5	19,41
19,24	0,81 (3H, d, j= 5,4 Hz)		36,3	36,54
34,72	1,74 (2H, m)		18,9	19,07
26,10	1,48 (2H, m)		34,0	34,00
46,74	0,97 (1H, m)	H-27	29,3	26,16
32,55	1,55 (1H, m)		45,9	45,89
19,82	0,88 (3H, d, j= 2 Hz)		26,2	29,23
20,10	0,85 (3H, d, j= 2,9 Hz)		19,1	19,83
23,77	1,31 (2H, m)		20,0	18,81
12,60	0,79 (3H, m)		23,2	23,12

Conclusion

The 11,12-dehydro-β-sitosterol compound has been isolated from the S. pictus Hassk stem in the shape of a needle crystal with a melting point of 132-134oC.

Acknowledgements

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References

Asih, N. P. S., Tri, W., & Agung, K. (2015). Studi inventarisasi Araceae di Gunung Seraya (Lempuyang), Karangasem, Bali. *Pros Sem Nas Masy Biodiv Indon*, *1*(3).

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Atun, S. (2014). Metode isolasi dan identifikasi struktur senyawa organik bahan alam. *Jurnal Konservasi Cagar Budaya Borobudur, 8*(2).

Dong, H., Yanling, G., Xueyong, W., Xiangyun, S., Xiao, W., & Jinqian, Y. (2018). Chemical constituents from *Scindapsus officinalis* (Roxb.) Schott. and their anti-inflammatory activities. *Molecules, 23*, 2577.

Hutapea, J. R. (2000). *Inventaris tanaman obat Indonesia (I)*. Jakarta: Badan Penelitian dan Pengembangan Kesehatan, Departemen Kesehatan RI.

Jumadi, O., Pince, S., Ramly, & Raoda. (2012). Riset khusus eksplorasi pengetahuan lokal etnomedisin dan tumbuhan obat di Indonesia berbasis komunitas. Kerjasama antara Badan Penelitian & Pengembangan Kesehatan Kementrian Kesehatan RI dengan Lembaga Penelitian Universitas Negeri Makassar.

Kaur, K., & Rajiv, G. (2016). Ethnobotanical and phytopharmacological review of *Scindapsus officinalis* ("Gajapippali"). *Asian Pacific Journal of Tropical Biomedicine*.

Mamahit, L. (2009). Eikodekana dari daun tumbuhan gedi (*Abelmoschus manihot L. Medik*) asal Sulawesi Utara. *Chemistry Progress, 2*(2).

Salempa, P., Noor, A., Soekamto, N. H., & Harlim, T. (2009). Bioaktivitas fraksi n-heksan dan senyawa β-sitosterol dari kayu akar *Pterospermum subpeltatum* C. B. Robb. *Farmakologi, 4*(2).

Sastrohamidjojo, H. (1992). *Spektroskopi inframerah*. Yogyakarta: Liberty Yogyakarta.

Syamsiah. (2016). *Taksonomi tumbuhan tinggi*. Makassar: Jurusan Biologi FMIPA UNM.

Wijaya, B. A., Gayatri, C., & Frenly, W. (2014). Potensi ekstrak etanol tangkai daun talas (*Colocasia esculenta [L]*) sebagai alternatif obat luka pada kulit kelinci (*Oryctolagus cuniculus*). *Pharmacon, 3*(3).

Yuzammi, & Reza, R. R. (2015). *Scindapsus splendidus* Alderw.: 'Living type' yang tersisa koleksi Kebun Raya Bogor. *Warta Kebun Raya, 13*(2).

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