Original Article

Isolation of lupeol acetate from fruit peels of *Artocarpus camansi*

Rosnani Nasution, Nurul Muhabbah, Hira Helwati, Muhammad Bahi, Marianne Marianne[1], Ulil Amna[2]

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, [1]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, [2]Department of Chemistry, Faculty of Engineering, Universitas Samudra, Langsa, Indonesia

J. Adv. Pharm. Technol. Res.

Abstract

The purpose of this research is to find a lupeol acetate from *Artocarpus camansi* fruit peel. Ethyl acetate extract of *A. camansi* fruit peel was obtained by maceration process. After the process of fractionation, it results 3 subfractions (A, B, and C). The subfraction B was rechromatographed and yielded $B2_2$ pure isolate. Based on data from proton nuclear magnetic resonance, Fourier transform–infrared, and mass spectrometry (MS from gas chromatography-MS), the $B2_2$ isolate was suspected as lupeol acetate compound (in this study, the presence of lupeol acetate in the *A. camansi* fruit peel has been reported for the first time).

Key words: Lupeol acetate, *Artocarpus camansi,* isolation

INTRODUCTION

Artocarpus camansi is known as breadnut (English), castana (Spanish), kamansi, kolo, pakau, ugod (Philippines), kelur, kulor, kulur, curor (Malaya, Java), and others. In Indonesia, the *A. camansi* plant is often referred as kulu or kluih. The plant of *A. camansi* is very similar to *Artocarpus communis* (breadfruit). A marked difference between the plant of *A. camansi* and the plant of *A. communis* is found in several parts, such as fruit, where *A. camansi* fruit has fine spines and seeds, while breadfruit (*A. communis*) has no seeds and does not have real fine spines on the fruit.[1]

Research on plants of *A. camansi* is relatively still rarely both in its activity and chemical compounds. However, the research on *A. communis*, the similar plants to *A. Camansi,* was relatively completed.[1] Our research reported that *A. camansi* plant leaf produced β-sitosterol propionate compound, which is lowering blood glucose.[2] Further studies of *n*-hexane extract of *A. camansi* bark contained β-amyrin acetate and Cycloeugenol and cycloeucalenol acetate that actively lower blood glucose.[3] The ethyl acetate extract of *A. camansi* bark contained β-sitosterol which actively lowered blood glucose.[4] Research on dichloromethane extract of *A. camansi* leaf has also been done, and it contained friedelinol, squalene, β-sitosterol, stigmasterol, and phytol; while, its bark contained polyprenol, cycloartenol, and cycloartenol acetate.[5] Although some of the compounds from *A. camansi* leaf and bark had been known, we need to study the fruit of *A. camansi* plant to obtain the chemical compounds that are likely similar to the leaves and barks.

Address for correspondence:
Prof. Rosnani Nasution,
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala,
Banda Aceh 23111, Indonesia.
E-mail: rosnani@unsyiah.ac.id

Submitted: 16-Jan-2020 **Revised:** 12-Mar-2020
Accepted: 06-Apr-2020 **Published**: 14-Jul-2020

Access this article online
Quick Response Code:
Website: www.japtr.org
DOI: 10.4103/japtr.JAPTR_6_20

SUBJECTS AND METHODS

Plant materials and bioindicators

The sample used in this research is *A. camansi* fruit peel collected in 2018, from Aceh Besar, Aceh, Indonesia. The

How to cite this article: Nasution R, Muhabbah N, Helwati H, Bahi M, Marianne M, Amna U. Isolation of lupeol acetate from fruit peels of *Artocarpus camansi*. J Adv Pharm Technol Res 2020;11:148-56.

plant was identified at Herbarium Medanense, Department of Biology, Universitas Sumatera Utara, Medan.

Generals

Mass spectra were characterized using a Shimadzu gas chromatography–mass spectrometry (GC-MS) QP2010 Ultra. The 1D nuclear magnetic resonance (^{1}H-NMR) spectrum was measured in a $CDCl_3$ solvent with 400 MHz JEOL spectrophotometer. The infrared (IR) spectra were recorded on PerkinElmer Fourier transform-IR spectrophotometer, using a KBr disc in the range 4000–400 cm^{-1}. Column chromatography was performed on silica gel G60 (70–230 mesh Merck). Thin-layer chromatography (TLC) analysis was carried out using precoated silica gel G60-F_{254} on aluminum foil (Merck).

Phytochemical screening

The method used for testing the phytochemicals can be found in phytochemical methods, a guide to modern techniques of plant analysis. Testing of triterpenoids is with the Liebermann–Burchard reagent (anhydrous acetic acid and concentrated sulfuric acid), phenolic testing with ferric chloride reagent.[6]

Extraction of *Artocarpus camansi* fruit peels

As much as 30 g of ethyl acetate extract was fractionated using gravity column chromatography. The eluent system used was *n*-hexane:ethyl acetate with a ratio of 8:2; 7:3; and 5:5, obtained as many as 95 fractions. All fractions are monitored by TLC. The fractions that have the same mode pattern are combined to obtain three subfractions (A, B, and C). The subfractions of the extract of ethyl acetate of the *A. camans*i fruit peels are shown in Table 1.

Based on the results of the grouping in Table 1, further separation is focused on subfraction B because it is cleaner, then sub-fraction B as much as 2.18 g is separated by the column Chromatography with n-hexane eluent: ethyl acetate (8: 2) and produced 23 fractions which can be seen in Figure 1.

From chromatogram in Figure 1, fractions 12 and 13 (subfraction B2) which are relatively pure are combined and obtained as much as 0.8 g, the B2 subfraction was rechromatographed again with eluent of *n*-hexane:ethyl acetate (7:3), and 23 fractions were obtained; the chromatogram of B2 subfraction is shown in Figure 2.

From chromatogram in Figure 2, fractions 10–21 (subfraction $B2_2$) which are relatively pure are combined and tested for their purity with three different eluents, *n*-hexane:ethyl acetate (a) 8:2 (b) 7:3 (c) 6:4. The yield of TLC chromatogram under ultraviolet light shows one stain pattern that indicated as pure compound. The chromatogram of subfraction $B2_2$ is shown in Figure 3.

The $B2_2$ isolated in Figure 3 shows a pattern of one stain. To confirm the structure, the isolate was then analyzed using ^{1}H-NMR and IR.

RESULTS AND DISCUSSIONS

Phytochemical test results

Phytochemical tests of *A. camansi* fruit peel showed secondary metabolites: triterpenoids, which were present in fresh samples, ethyl acetate extracts, subfractions A, B, C, and isolates of $B2_2$. The isolate of $B2_2$ showed a negative result to the ferric chloride test, and a positive result was observed to the Liebermann–Burchard test [Table 2], thus confirming that the compound is a steroid/triterpenoid type.

Characterization of the ethyl acetate extract of *Artocarpus camansi* fruit peels using gas chromatography–mass spectrometry

Ethyl acetate extract of *A. camansi* fruit peel was characterized using GC-MS. The results of GC are shown in Figure 4, while the results of characterization with MS, and after being

Table 1: Subfraction of the extract of ethyl acetate of the *Artocarpus camansi* fruit peels

Fraction	Subfraction	Weight (g)	Characteristic features
A	1-48	30,979	Gel/black
B	49-66	24,929	Gel/black
C	67-95	38,273	Gel/black

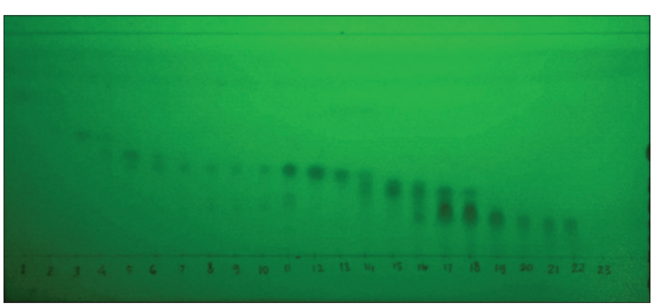


Figure 1: The chromatogram of fractions from subfraction B with eluent of *n*-hexane:ethyl acetate (8:2)

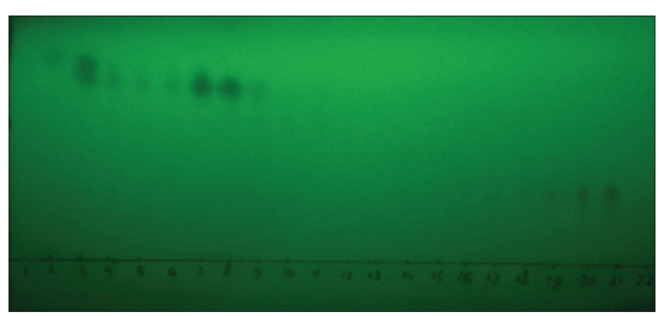


Figure 2: The chromatogram of fractions from subfraction B2 with eluent of *n*-hexane:ethyl acetate (7:3)

analyzed based on Library: NIST14.lib data on MS, the compounds are shown in Table 3.

Based on the chromatogram in Figure 4 and characterization with MS, it was found that the ethyl acetate extract of *A. camansi* fruit peel contained 25 compounds. These compounds are shown in Table 3.

The area and peak height in GC can be used for quantitative analysis so that the levels of each compound can be determined.[7] Based on Table 3, there are several compounds that have a wide area and a high peak, which makes it possible to be isolated because these compounds are obtained at high levels, such as zonarone (57.85%), and 9,19-Cyclolanost-24-en-3-ol (12.71%).

Table 2: Secondary metabolite test on B2$_2$ isolates

Pure isolate	Test	Result	Inference
B2$_2$	Ferric chloride	−	Nonphenolic
	Liebermann	+	Terpenoid/steroid

+: Positive, −: Negative

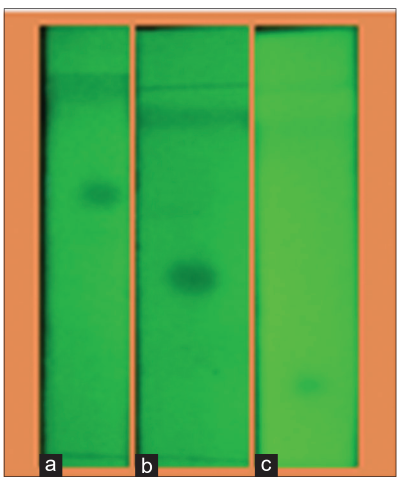


Figure 3: The chromatogram of isolates of B2$_2$ with eluent of *n*-hexane:ethyl acetate (a) 6:4, (b) 7:3, and (c) 8:2

The composition of compounds in *A. camansi* fruit peel extract contains, among others, straight and cyclic chains. Cyclic compounds are generally as terpenoid, monoterpenoid (trans-geraniol), and triterpenoid (methyl commate C; 9,19-Cyclolanost-24-en-3-ol; 9,19-Cyclolanost-23-ene-3,25-diol, 3-acetate), and steroids (Spiro androst-5-ene-17,1′-cyclobutane-2′-one, 3-hydroxy; DELTA.5-Ergostenol (δ.5-ergostenol); trans-stigmasta-5,22-dien-3 β.-ol). For compounds with high similarities with compounds in library MS, it is very close to the actual compound.

Characterization the isolate B2$_2$ (lupeol acetate)

The B2$_2$ isolates were characterized using ^{1}H-NMR, and the results of characterization are shown in Figure 5.

Based on the ^{1}H-NMR spectrum, the signal showed 8 methyls at δH 2.04 (3H, σ, H– 2э), 1.74 (3H, σ, H–30), 0.97 (3H, σ, H–25), 0.89 (3H,σ, H–28), 0.88 (3H, σ, H–23), 0.85 (3H, σ, H–24), 0.84 (3H, σ, H–26), δαν 0.83 ππμ (3H, σ, H–27). Doublet at 4.85 and 4.94 ppm shows C-29 atomic shifts (2H, dd, H-29a and H-29b) and methyl singlets at 1.74 for C-30, indicating that the B2$_2$ isolate is triterpenoid type.[8] The multiplet at δH 4.31 ppm is a typical signal for proton C-3 α-orientation and δH 2.04 ppm, for C 2′, indicating that the B2$_2$ isolate is a triterpenoid derived from lupeol ester.[8]

The ^{1}H-NMR spectrum for aliphatic protons (CH_3, CH_2, and CH) in triterpenoid compounds is usually seen in the chemical shift (δH) 2 ppm. Aliphatic protons are cyclic protons from the basic triterpenoid framework that are not well separated.[9]

Based on the above reason, the isolate B2$_2$ as ester lupeol compared with lupeol acetate compound. A comparison of the chemical shift of B2$_2$ isolate compound with lupeol acetate compound is shown in Table 4.

Table 4 shows that there is a proton that absorbs around δH1.5 ppm like protons at H-1, H-2, H-5, H-6, and H-7

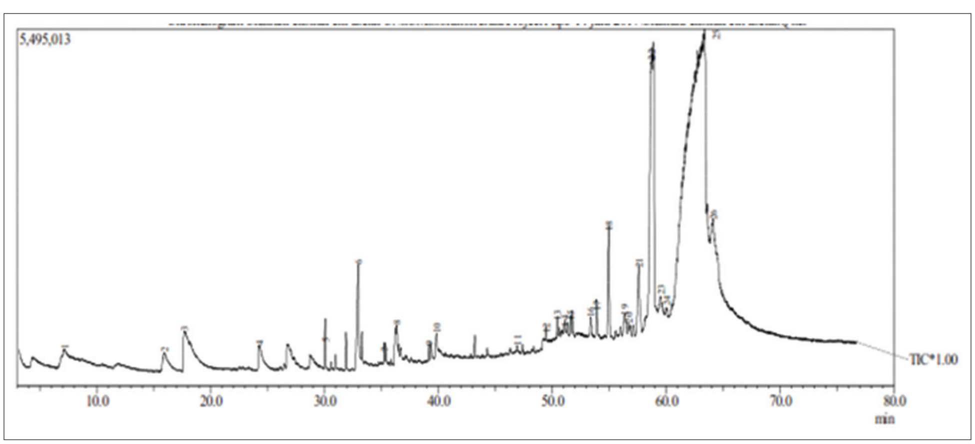
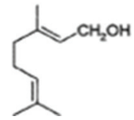
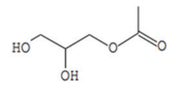
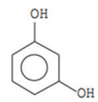
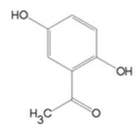
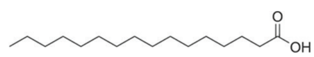
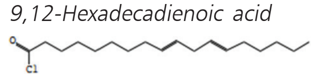
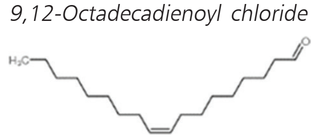
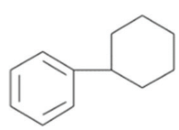
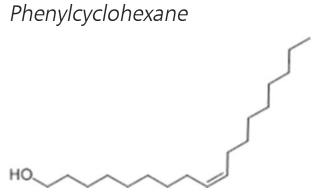
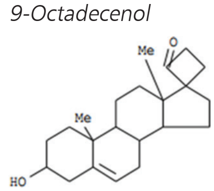


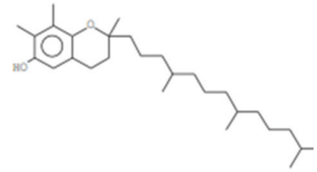
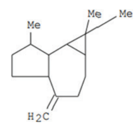
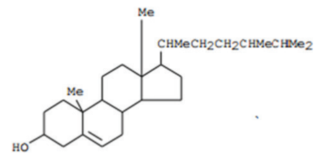
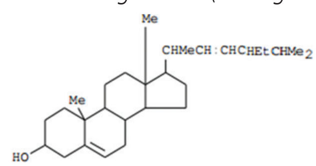
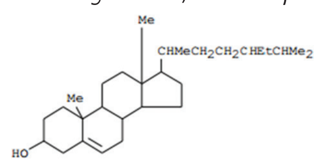
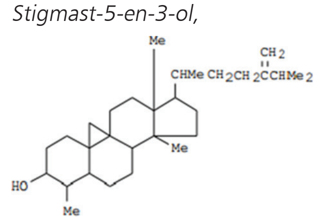
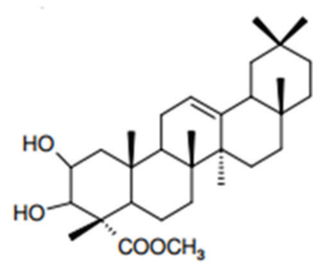
Figure 4: Chromatogram of ethyl acetate extract of *A. camansi* fruit peel by gas chromatography

Table 3: The compounds contained in the ethyl acetate extract of *Artocarpus camansi* fruit peel (characterization by gas chromatography-mass spectrometry)

Peak	Retention time	Area (%)	Name of compound	Similarity (%)
1	7.203	0.66	 *Trans-geraniol*	85
2	15.974	1.05	 *1,2,3-Propanetriol, 1-acetate*	92
3	17.542	3.72	 *Resorcinol*	94
4	24.297	1.14	 *Ethanone*	91
5	30.083	1.24	 *Octadecanal*	89
6	32.977	2.29	 *Palmitic acid*	94
7	35.286	0.33	 *9,12-Hexadecadienoic acid*	91
8	38.378	0,2	 *9,12-Octadecadienoyl chloride*	90
9	39.00	0.36	 *9-Octadecenal*	83
10	39.846	0.69	 *Phenylcyclohexane*	63
11	46.948	0.14	 *9-Octadecenol*	77
12	49.492	0.91	 *Spiro androst-5-ene-17,1'-cyclobutan-2'-one, 3-hydroxy*	69

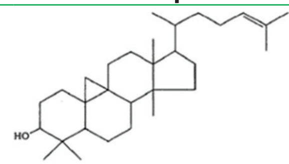
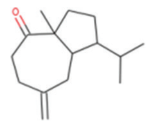
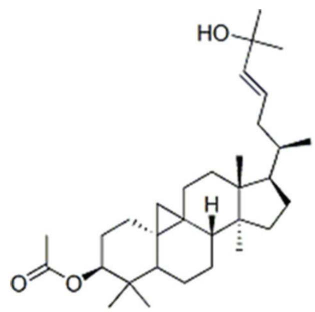
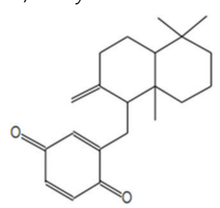
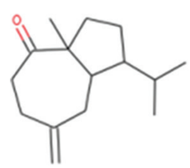
Contd...

Table 3: Contd...

Peak	Retention time	Area (%)	Name of compound	Similarity (%)
13	50.461	0.39	 *γ-Tocopherol*	77
14	51.142	0.96	*Tetratetracontane*	80
15	51.625	0.47	 *Aromadendrene*	82
16	53.399	0.30	 *DELTA.5-Ergostenol (δ.5-ergostenol)*	91
17	53.942	0.52	 *Trans-stigmasta-5,22-dien-3β.-ol*	89
18	54.981	1.72	 *Stigmast-5-en-3-ol,*	90
19	56.359	1.69	 *Cycloeucalenol*	86
20	57.617	1.60	 *Methyl commate C*	82

Contd...

Table 3: Contd...

Peak	Retention time	Area (%)	Name of compound	Similarity (%)
21	58.748	12.71	 *9,19-Cyclolanost-24-en-3-ol*	84
22	59.523	1.36	 *Salvial-4 (14)-en-1-one*	73
23	60.042	0.46	 *9,19-Cyclolanost-23-ene-3,25-diol, 3-acetate*	80
24	63.371	57.85	 *Zonarone*	67
25	64.133	7.10	 *Salvial-4 (14)-en-1-one*	67

atoms. The proton at H-3 absorbs more below the field at δ = 4.13 ppm as it is influenced by the O atom having a large electronegativity.[11] The lupeol acetate compound has a double bond on H-29, thus causing absorbing absorption at δ = 4.31 ppm and δ = 4.85 ppm. This is because the proton is attached to a carbon sp2 (C = C).[11]

Based on the above data, the $B2_2$ isolate suspected to be lupeol acetate compounds. This is confirmed by the presence of the MS spectrum which shows a similarity in the fragmentation pattern of $B2_2$ isolates with lupeol acetate compounds. The spectrum of the mass spectrometry of the isolate $B2_2$ is shown in Figure 6.

The mass spectrometry of the $B2_2$ spectrum shows the peaks of m/z: 453, 408, and 393 and peaks at 218, 203, and 189, as well as the peak base at m/e 43 (100%), showing fragmentation patterns similar to compounds of lupeol acetate.[12]

The presence of acetate groups in the lupeol compound was amplified by the presence of carbonyl uptake (C = O) at the wave number at 1716 cm^{-1} and the absorption of CO at wave number of 1248 cm^{-1} at IR [Figure 7].

The ester of the compound was studied has a band at 1640 cm^{-1} indicated C = C vibrations, (C29), and C-O band found in the fingerprint area 1110–1300 cm^{-1}[13] so that the $B2_2$ isolates were predicted as lupeol acetate [Figure 8].

From the results of GC-MS, the abundant compound in ethyl acetate extract was 9,19-Cyclolanost-24-en-3-ol (area: 12.71%; retention time: 57,748 min; and similarity: 84%), and this compound is a triterpenoid. Besides the presence of zonarone (retention time of 63,371 min and area of 57.85%; similarity of 67%), its structure as the initial skeletal precursor of lupan (the lupeol acetate has lupan framework). Of the two compounds present in GC-MS, this

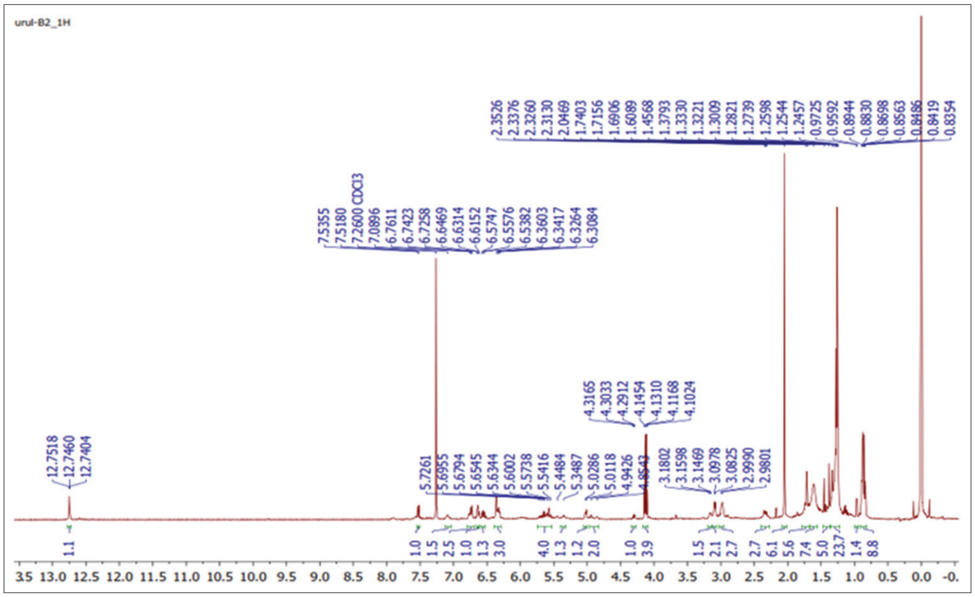


Figure 5: The spectrum of [1]H-nuclear magnetic resonance of $B2_2$ isolate

Table 4: Comparison of δ proton nuclear magnetic resonance of the isolated B22 with lupeol acetate of standard

The position of atom H	Isolate B22	lupeol acetate standard	The position of atom H	Isolate B22	lupeol acetate standard
1	1.60 m 1.72 m	1.51 m 1.72 m	16	1.42 m 1.60 m	1.42 m 1.56 m
2	1.60 m 1.71 m	1.50 m 1.70 m	17	-	-
3	4.31m	4.48 dd	18	1.60 m	1.56 m
4		-	19	2.35 m	2.37 dt
5	0.83 m	0.70 m	20	-	-
6	1.44 m 1.59 m	1.44 m 1.53 m	21	0.86 m 1.08 m	0.87 m 1.08 m
7	1.25 m 1.49 m	1.25 m 1.49 m	22	1.33 m 1.45 m	1.33 m 1.46 m
8	-	-	23	0.88 s	0.85 s
9	1.30 m	1.30 m	24	0.85 s	0.88 s
10	-	-	25	0.97 s	0.88 s
11	1.25 m 1.47 m	1.25 m 1.47 m	26	0.84 s	1.03 s
			27	0.83 s	0.94 s
12	1.51 m 1.60 m	1.51 m 1.61 m	28	0.89 s	0.79 s
			29	4.85 m 4.94 d	4.57 m 4.69 d
13	1.63 m	1.63 m			
14	-	-	30	1.74 s	1.68 s
15	1.13 m 1.37 m	1.13 m 1.41 m	2′	2.04s	

Based on the above reason, the isolate B22 as ester lupeol compared with lupeol acetate of the standard[10]

has a relatively large similarity, as a framework supporting the skeleton of lupan so that the compound $B2_2$ is lupeol acetate which is an abundant compound in the ethyl acetate extract of *A. camansi* fruit peel.

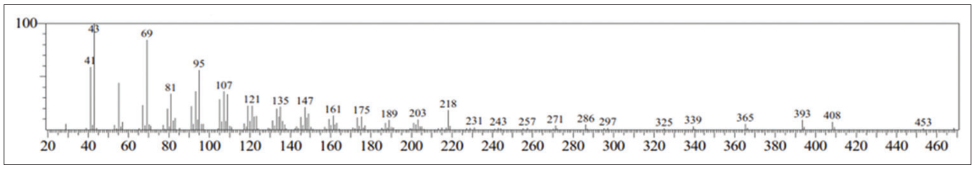


Figure 6: The spectrum of mass spectrometry of the isolated $B2_2$

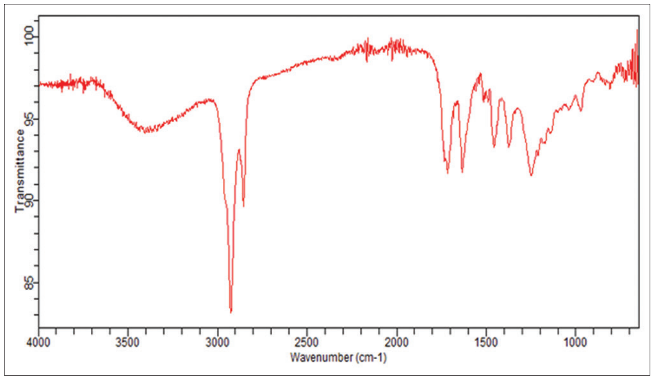


Figure 7: The infrared spectrum of the $B2_2$ isolate

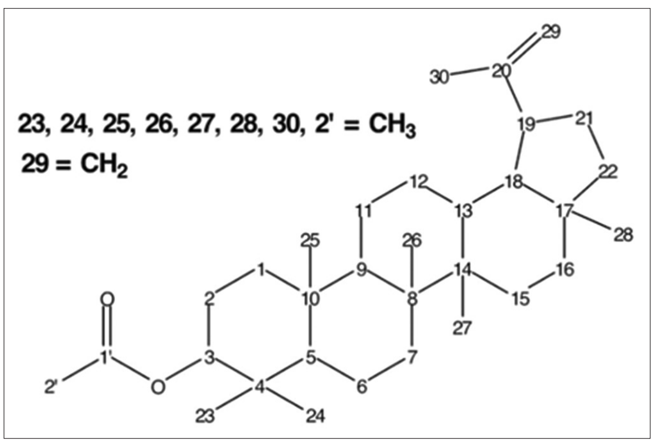


Figure 8: Structure of lupeol acetate compound[13]

A literature shows that secondary metabolites isolated from plants *A. camansi* are groups of terpenoids or steroid, which have one-way pathways, So it is very relevant to find terpenoid compounds in the peel of *A. camansi* fruit because, in other parts of this plant, steroids have been found. Both of these secondary metabolites have a one-way biosynthetic pathway. The structure of the lupeol acetate is included in the skeleton lupan-type triterpene, and the presence of lupeol is reported for the first time in the *A. camansi* peels.

Based on the literature, it is known that lupeol acetate has many benefits, including antinociceptive and anti-inflammatory.[14-16] Lupeol acetate also has an antimicrobial, anti-inflammatory, antimalarial, and antituberculosis activity.[17-19] Lupeol compounds can reduce the activity of α-amylase[20] and can inhibit Tyrosine phosphatase 1B.[21] In addition, lupeol also showed moderate inhibitory activity against glutathione S-transferase and acetylcholinesterase. Umbelliferone and lupeol studies (100 and 200 mg/kg BW) of banana flowers decreased fasting hyperglycemia activity in diabetic rats given for 4 weeks.[22]

CONCLUSIONS

Based on the phytochemical test, secondary metabolite in ethyl acetate extract of *A. camansi* fruit peel is triterpenoid. Characterization by GC-MS and ethyl acetate extract of *A. camansi* fruit contained 25 chemical compounds with the main compounds of zonaron (retention time: 63,371 min; area of 57.85%; and similarity: 67%) and 9,19-Cyclolanost-24-en-3-ol (area: 12.71%; retention time: 57,748 min, 84%: similarity). The results of B subfraction separation by column chromatography obtained isolate $B2_2$, based on spectra analysis of ^{1}H-NMR, IR, and MS, the isolate $B2_2$ was suspected as a lupeol acetate compound. In this study, the presence of lupeol acetate ($B2_2$) has been reported for the first time.

Acknowledgment

The author would like to thank Universitas Syiah Kuala, Ministry of Research, Technology and Higher Education in accordance with the Letter of Agreement for Research of Professor Candidates.

Financial support and sponsorship

This stud was financially supported by the Ministry of Research, Technology and Higher Education.

Conflicts of interest

There are no conflicts of interest.

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