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**ISOLATION AND MS STUDY OF FRIEDELINOL FROM THE LEAVES OF *TERAP*
(*ARTOCARPUS ODORATISSIMUS* BLANCO)**

*ERWIN¹, SRI SULISTYANINGSIH¹, AND IRAWAN W.KUSUMA²

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ABSTRACT.

Artocarpus odoratissimus Blanco (local name: *Terap*) is one of the *Artocarpus* species ~~collected was taken~~ from the tropical forests of East Kalimantan. ~~From leaves extracts of this plant, a triterpene, friedelinol has been isolated.~~ A friedelinol (triterpene) was isolated from the leaves extracts of the plant and the compound structure was ~~The structure of the compound~~ was established from the FT-IR and mass spectral data and confirmed by a standard compound. The compound ~~possessed~~ has moderate cytotoxicity against a brine shrimp, *A. salina* with LC₅₀ 48.39 ppm.

KEYWORDS: *Artocarpus*, *Terap*, *friedelinol*.

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INTRODUCTION

Moraceae is a large plant family consists of 60 genera and 1400 species, three of them ~~which~~ include a large genus : they are that is, *Artocarpus*, *Morus*, and *Ficus*[1]. *Artocarpus*, one of the main genus of Moraceae consisting of 50 species and spread distributed on tropical and sub-tropical region from South Asia, Southeast Asia to the Solomon Islands, Pacific Islands, North Australia and Central America [2,3,4]. Economically, the genus is of appreciable importance as a source of edible fruit, and yield fairly good timber [5]. *Artocarpus* typically grows as large and tall trees. The wood can be used as building materials, home furnishings, and bridges. In Indonesia, since the first several of *Artocarpus* species are widely used in folk medicines as root decoction of *Artocarpus integra* Merr finely used to treat fever [6].

Various species of this plant has been creating created as a traditional medicine known as *jamu* (Indonesia) [7]. In Borneo, there are 25 species, and of which 13 species are endemic, but only two species are used [3]. Several species of *Artocarpus* -many produce many triterpene, terpenoids, steroids, and phenolics including: flavonoids, stilbenoid, arilbenzofuran, jacalin, and Diels-Alder addition compounds [8].

The unique structure of secondary metabolites in *Artocarpus* produce a very wide effect, such as anti-bacterial [9] anti-platelet [10], antifungal [11], antimalarial [12,13], toxic and cytotoxic [14,15,16,17,18], and antidiabetic [3]. *Artocarpus odoratissimus* Blanco is one of the *Artocarpus* species was found as endemic species in tropical forests of Kalimantan (Borneo), Indonesia [19]. Traditionally, local people used leaves of *A. odoratissimus* as diabetic-treating drug and ash obtained from leaves is used as an antidote for centipede bite and scorpion stings, while the wood and bark were can-be used as an anti-malarial [20,21]. in Sumatera (Indonesia), *A. odoratissimus* used as a natural pesticide to repel mice [22]. Fruits and their by-products are rich sources of antioxidant, with significant high level of phenolic and flavonoid content[23].

Previous studies on *A. odoratissimus* reported the availability of artosimmin, traxateryl acetate and anthocyanin [23,24]. Our further work on the genus *Artocarpus* was to investigate the leaves of *A. odoratissimus* and to elucidate one of the molecular structure ~~one of~~ of triterpene type compounds.

MATERIALS AND METHODS

Materials

The leaves of of *A. odoratissimus* were collected from Sungai Siring, Samarinda city, East Kalimantan. The plant was identified by a staff at the Laboratory of Physiology, Mulawarman University. The silica gel used for flash column chromatography was a silica gl 60, 230-400 mesh (Merck, Darmstad-Germany). Thin layer chromatography aluminium sheets (Silica gel 60 F254, 0,25 mm) were purchased from Merck (Darmstadt, Germany). All other materials or solvents were of the highest purity or high-performance liquid chromatography (HPLC) grade.

Instrumentation

IR spectra were measured with PERKIN ELMER Spectrum one L.R. 64912C spectrophotometers. Mass spectra was were recorded on a Shimadzu GC-MS QP 5050A (Shimadzu Corp., Kyoto, Japan) at an electron energy of 70 eV (direct inlet). Melting point of

the compound was measured **by** using Yanaco micro melting point apparatus (Yanaco Co., Ltd., Kyoto, Japan) and were uncorrected.

Extraction

The dried powder of **the plant's** leaves (3 kg) **were was** macerated with methanol for 24 h at room temperature and repeated 4 times. The methanol extract obtained was **then** concentrated by a rotary vacuum evaporator to yield 40.19 g of total extract. Furthermore, the total extract was fractionated with *n*-hexane and concentrated **by** using a rotary vacuum evaporator to obtain 18.95 g of solid.

Isolation and purification

n-Hexane fraction from the methanol extract of *A. odoratissimus* leaves was subjected to a flash column chromatography over silica gel using the *n*-hexane and ethyl acetate in increasing polarity. The column chromatography yielded 50 tubes fractions that were pooled together based on their polarity into 4 fractions, fraction A (tubes 1-7, 190 mg), fraction B (tubes 8-15, 410 mg), fraction C (tubes 16-22, 1000 mg), and fraction D (tubes 23-50, 2790 mg). Fraction B was in the form of white crystals. Ten milligrams of the crystal B was recrystallized **by** using hot *n*-hexane to yield 6 mg of the pure compound **1**.

Phytochemical test

1 mg of compound **1** was put into a test tube following by addition of 2 drops of anhydride acetate and 1 drop of sulfuric acid (Liebermann-Burchard reagent). The formation of green indicated of the occurrence of steroid.

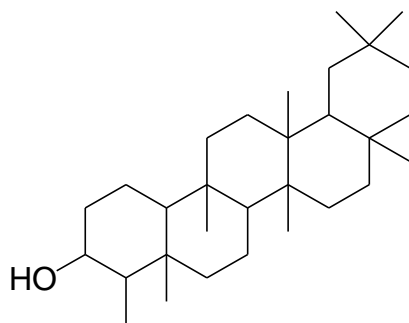
Biological assay

Compound **1** was tested for the toxicity by the brine shrimp lethality test against *Artemia salina* using previous report [25].

RESULT AND DISCUSSION

Compound **1** was obtained as a white powder having melting point at 285 - 287°C. **It showed showing** positive reaction with Liebermann Burchard reagent indication of terpenoid. Mass spectral data showed a peak at m/z 428 (M^+) that in accordance with molecular formula $C_{30}H_{52}O$. IR spectral data showed maximum absorbance at 3479.58 cm^{-1} , suggested stretching absorption of hydroxyl group. This suggestion was supported by appearance of bending absorption of secondary cyclic C-OH at 1018.41 cm^{-1} . Peaks with sharp intensity at 2931.8 cm^{-1} and 2870.08 cm^{-1} showed a C-H stretching of CH_3 and CH_2 , that **was is** supported by a bending at 1388.75 cm^{-1} . A peak with sharp intensity at 1705.07 cm^{-1} suggested the occurrence of non conjugated C=O. Peak at 1635.64 cm^{-1} suggested the presence of non conjugated C=C. A finger print area at 1172.72 cm^{-1} suggested the presence of C-O bond. Based on infra red spectral data,

it ~~was can be~~ concluded that compound 1 consisted hydroxyl, CH₃ and CH₂, C=O, C=C double bond and C-O groups.



(1)

Figure 1. Friedelinol

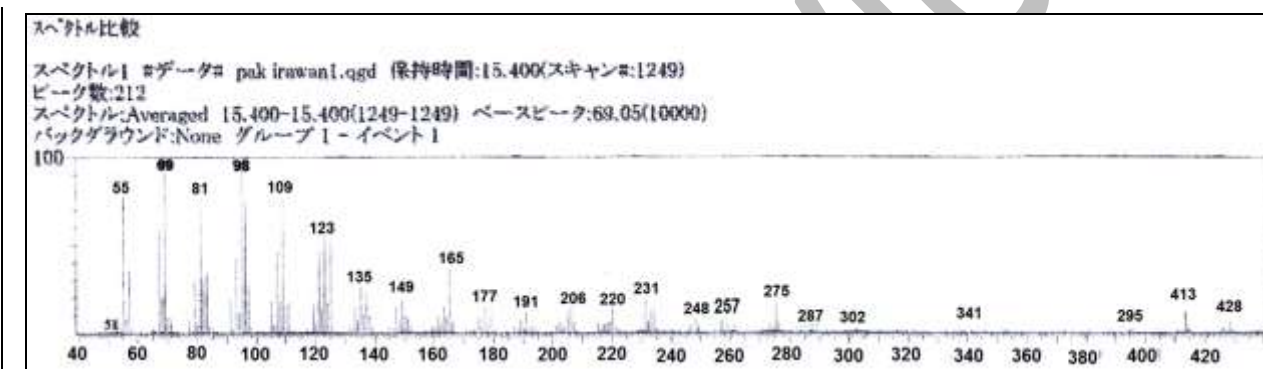


Figure 2. MS spectrum of friedelinol

Tabel 1. Comparison of Mass Fragmentation of Compound 1 and Friedelinol.

No	Mass spectra fragmentation pattern	
	Compound 1 (<i>m/z</i>)	Friedelinol (<i>m/z</i>)
1	428	428
2	413	413
3	382	-
4	341	-
5	275	275
6	257	257
7	248	248
8	231	231
9	220	220

10	206	206
11	191	189
12	177	177
13	165	165
14	149	147
15	135	137
16	123	125
17	109	109
18	98	98
19	81	81
20	55	55

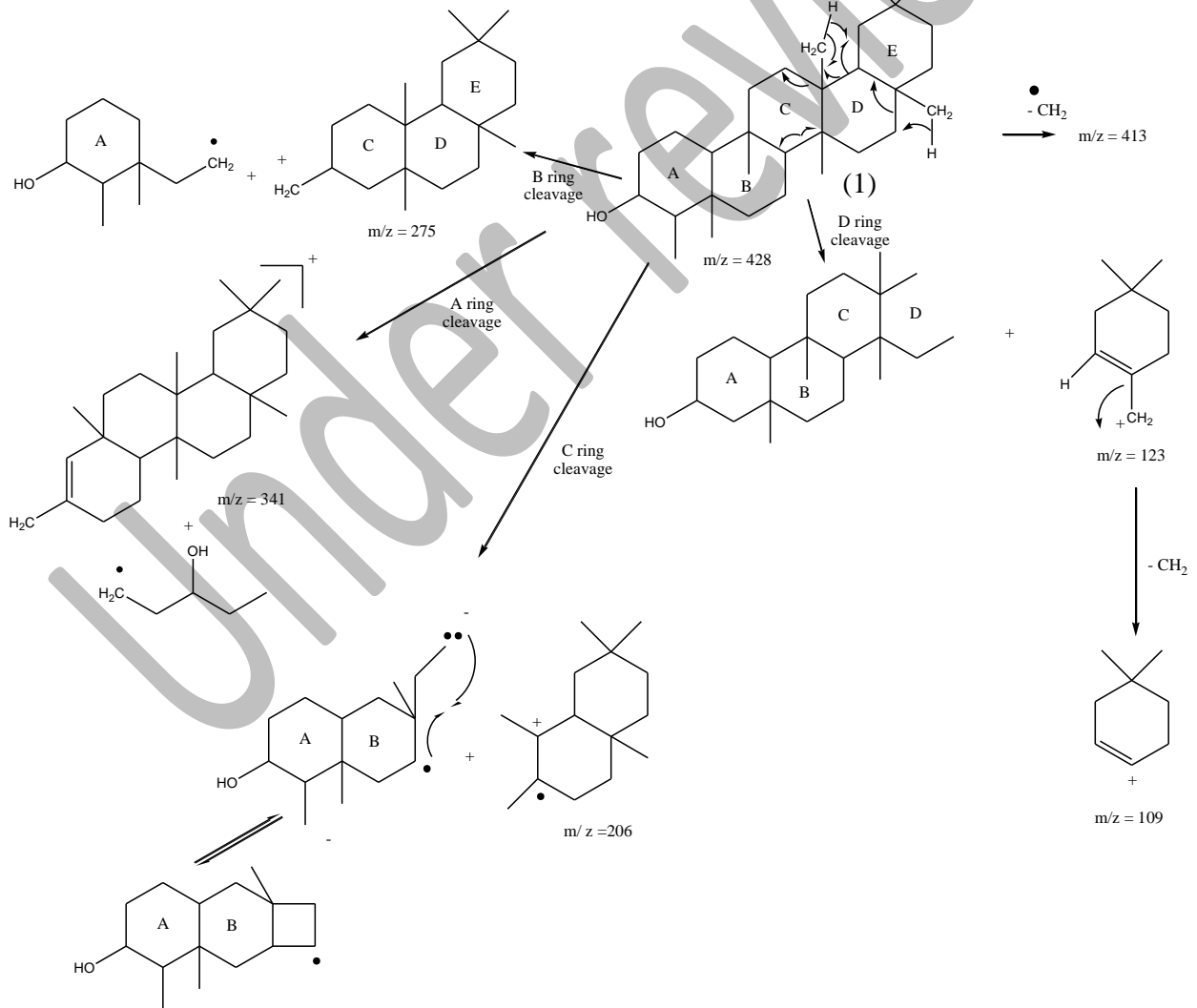


Figure 3. Mass Fragmentation Pattern of Compound 1.

The result of GC-MS analysis displayed a molecular peak at m/z 428 (M^+) suggested the molecular weight of compound **1**, that matched to the molecular formula $C_{30}H_{52}O$. Fragmentation pattern of compound **1** was as presented at Fig 1 and indicated the possibility of the compound **1** as a triterpene, friedelinol. Comparison of mass spectral fragmentation pattern of compound **1** to that of friedelinol was as presented at Table 1, it showed a good match. Based on the description of physicochemical properties, therefore, the compound **1** was identified as friedelinol.

CONCLUSION

Based on the spectroscopical analysis of FT-IR and GC-MS, an isolated compound from *A. odoratissimus* was identified as triterpene, friedelinol. The compound has possessed moderate cytotoxicity against a brine shrimp, *A. salina* with LC_{50} 48.39 ppm.

ACKNOWLEDGEMENT

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**ISOLATION AND MS STUDY OF FRIEDELINOL FROM THE LEAVES OF *TERAP*
(*ARTOCARPUS ODORATISSIMUS* BLANCO)**

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ABSTRACT.

Artocarpus odoratissimus Blanco (local name: *Terap*) is one of the *Artocarpus* species was taken from the tropical forests of East Kalimantan. . A friedelinol (triterpene) was isolated from the leaves extracts of the plant and the compound structure was established from the FT-IR and mass spectral data and confirmed by a standard compound. The compound has moderate cytotoxicity against a brine shrimp, *A. salina* with LC₅₀ 48.39 ppm.

KEYWORDS: *Artocarpus*, *Terap*, *friedelinol*.

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INTRODUCTION

Moraceae is a large plant family consists of 60 genera and 1400 species, three of them include a large genus : they are , Artocarpus, Morus, and Fiscus[1]. *Artocarpus*, one of the main genus of Moraceae consisting of 50 species and spread on tropical and sub-tropical region from South Asia, Southeast Asia to the Solomon Islands, Pacific Islands, North Australia and Central America [2,3,4]. Economically, the genus is of appreciable importance as a source of edible fruit, and yield fairly good timber [5]. Artocarpus typically grows as large and tall trees. The wood can be used as building materials, home furnishings, and bridges. In Indonesia, the first several of Artocarpus species are widely used in folk medicines as root decoction of *Artocarpus integra* Merr finely used to treat fever [6].

Various species of this plant has been creating as a traditional medicine known as *jamu* (Indonesia) [7]. In Borneo, there are 25 species, and 13 species are endemic, but only two species are used [3]. Several species of Artocarpus produce many triterpene, terpenoids, steroids, and phenolics including; flavonoids, stilbenoid, arilbenzofuran, jacalin, and Diels-Alder addition compounds [8].

The unique structure of secondary metabolites in Artocarpus produce a very wide effect, such as anti-bacterial [9] anti-platelet [10], antifungal [11], antimalarial [12,13], toxic and cytotoxic [14,15,16,17,18], and antidiabetic [3]. *Artocarpus odoratissimus* Blanco is one of the *Artocarpus* species was found as endemic species in tropical forests of Kalimantan (Borneo), Indonesia [19]. Traditionally, local people used leaves of *A. odoratissimus* as diabetic-treating drug and ash obtained from leaves is used as an antidote for centipede bite and scorpion stings, while the wood and bark were used as an anti-malarial [20,21]. in Sumatera (Indonesia), *A. odoratissimus* used as a natural pesticide to repel mice [22]. Fruits and their by-products are rich sources of antioxidant, with significant high level of phenolic and flavonoid content[23].

Previous studies on *A. odoratissimus* reported the availability of artosimmin, traxateryl acetate and anthocyanin [23,24]. Our further work on the genus *Artocarpus* was to investigate the leaves of *A. odoratissimus* and to elucidate one of the molecular structure of triterpene type compounds.

MATERIALS AND METHODS

Materials

The leaves of *A. odoratissimus* were collected from Sungai Siring, Samarinda city, East Kalimantan. The plant was identified by a staff at the Laboratory of Physiology, Mulawarman University. The silica gel used for flash column chromatography was a silica gl 60, 230-400 mesh (Merck, Darmstad-Germany). Thin layer chromatography aluminium sheets (Silica gel 60 F254, 0,25 mm) were purchased from Merck (Darmstadt, Germany). All other materials or solvents were of the highest purity or high-performance liquid chromatography (HPLC) grade.

Instrumentation

IR spectra were measured with PERKIN ELMER Spectrum one L.R. 64912C spectrophotometers. Mass spectra was recorded on a Shimadzu GC-MS QP 5050A (Shimadzu Corp., Kyoto, Japan) at an electron energy of 70 eV (direct inlet). Melting point of the compound

was measured by using Yanaco micro melting point apparatus (Yanaco Co., Ltd., Kyoto, Japan) and were uncorrected.

Extraction

The dried powder of the plant's leaves (3 kg) were macerated with methanol for 24 h at room temperature and repeated 4 times. The methanol extract obtained was concentrated by a rotary vacuum evaporator to yield 40.19 g of total extract. Furthermore, the total extract was fractionated with *n*-hexane and concentrated by using a rotary vacuum evaporator to obtain 18.95 g of solid.

Isolation and purification

n-Hexane fraction from the methanol extract of *A. odoratissimus* leaves was subjected to a flash column chromatography over silica gel using the *n*-hexane and ethyl acetate in increasing polarity. The column chromatography yielded 50 tubes fractions that were pooled together based on their polarity into 4 fractions, fraction A (tubes 1-7, 190 mg), fraction B (tubes 8-15, 410 mg), fraction C (tubes 16-22, 1000 mg), and fraction D (tubes 23-50, 2790 mg). Fraction B was in the form of white crystals. Ten milligrams of the crystal B was recrystallized by using hot *n*-hexane to yield 6 mg of the pure compound **1**.

Phytochemical test

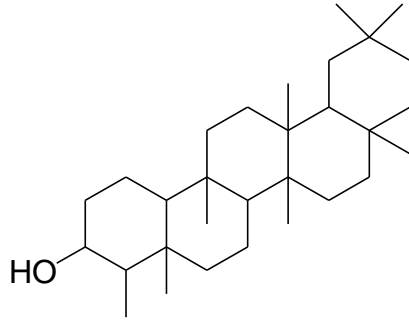
1 mg of compound **1** was put into a test tube following by addition of 2 drops of anhydride acetate and 1 drop of sulfuric acid (Liebermann-Burchard reagent). The formation of green indicated of the occurrence of steroid.

Biological assay

Compound **1** was tested for the toxicity by the brine shrimp lethality test against *Artemia salina* using previous report [25].

RESULT AND DISCUSSION

Compound **1** was obtained as a white powder having melting point at 285 - 287°C. It showed positive reaction with Liebermann Burchard reagent indication of terpenoid. Mass spectral data showed a peak at m/z 428 (M^+) that in accordance with molecular formula $C_{30}H_{52}O$. IR spectral data showed maximum absorbance at 3479.58 cm^{-1} , suggested stretching absorption of hydroxyl group. This suggestion was supported by appearance of bending absorption of secondary cyclic C-OH at 1018.41 cm^{-1} . Peaks with sharp intensity at 2931.8 cm^{-1} and 2870.08 cm^{-1} showed a C-H stretching of CH_3 and CH_2 , that was supported by a bending at 1388.75 cm^{-1} . A peak with sharp intensity at 1705.07 cm^{-1} suggested the occurrence of non conjugated C=O. Peak at 1635.64 cm^{-1} suggested the presence of non conjugated C=C. A finger print area at 1172.72 cm^{-1} suggested the presence of C-O bond. Based on infra red spectral data, it was concluded that compound **1** consisted hydroxyl, CH_3 and CH_2 , C=O, C=C double bond and C-O groups.



(1)

Figure 1. Friedelinol

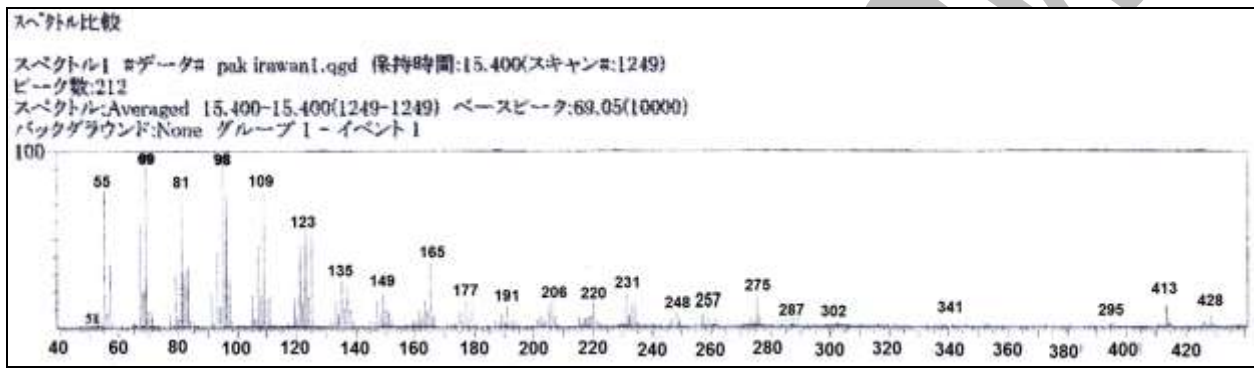


Figure 2. MS spectrum of friedelinol

Tabel 1. Comparison of Mass Fragmentation of Compound 1 and Friedelinol.

Mass spectra fragmentation pattern		
No	Compound 1 (<i>m/z</i>)	Friedelinol (<i>m/z</i>)
1	428	428
2	413	413
3	382	-
4	341	-
5	275	275
6	257	257
7	248	248
8	231	231
9	220	220
10	206	206
11	191	189
12	177	177

13	165	165
14	149	147
15	135	137
16	123	125
17	109	109
18	98	98
19	81	81
20	55	55

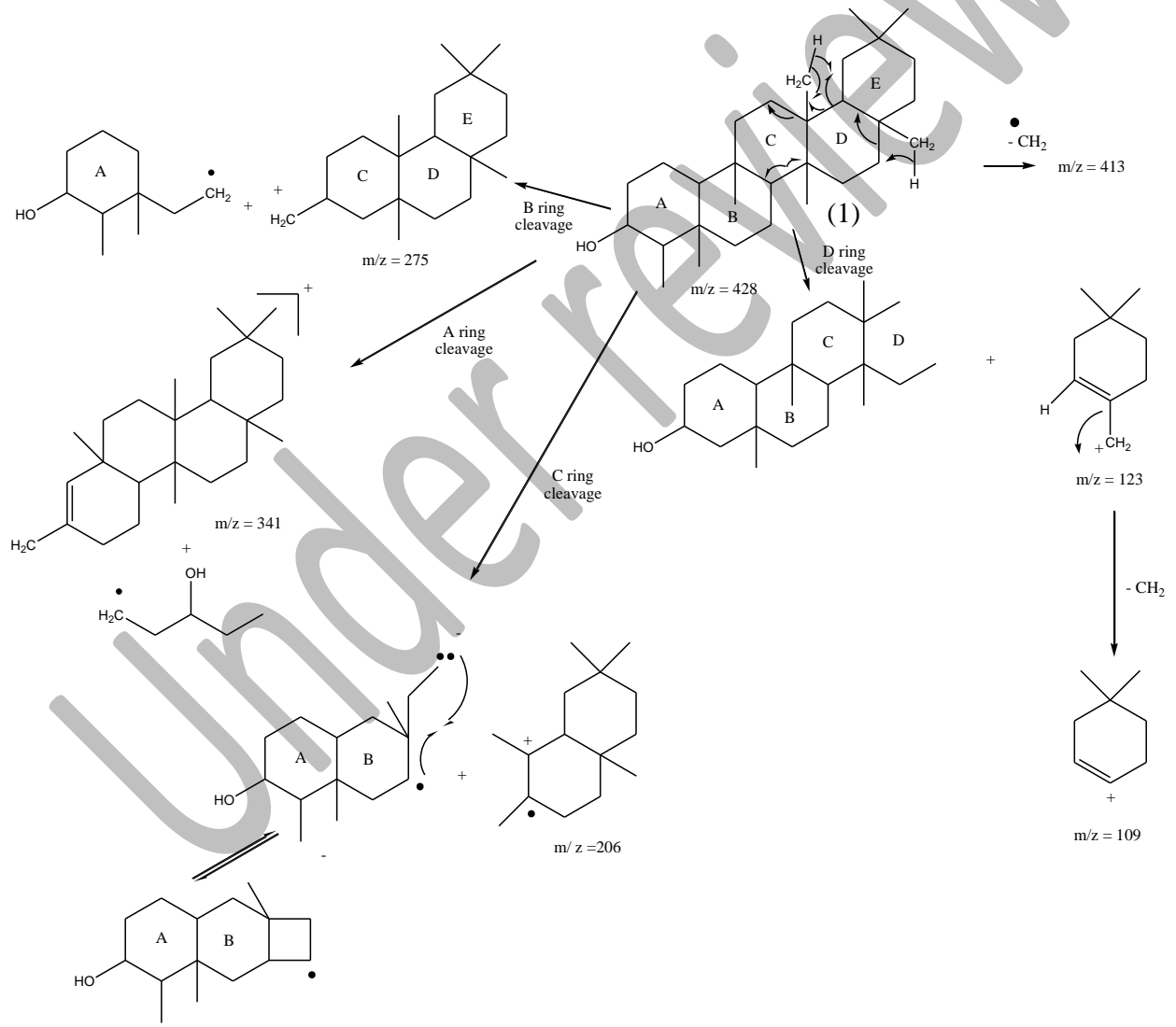


Figure 3. Mass Fragmentation Pattern of Compound 1.

The result of GC-MS analysis displayed a molecular peak at m/z 428 (M^+) suggested the molecular weight of compound **1**, that matched to the molecular formula $C_{30}H_{52}O$. Fragmentation pattern of compound **1** was presented at Fig 1 and indicated the possibility of the compound **1** as a triterpene, friedelinol. Comparison of mass spectral fragmentation pattern of compound **1** to that of friedelinol was presented at Table 1, it showed a good match. Based on the description of physicochemical properties, therefore, the compound **1** was identified as friedelinol.

CONCLUSION

Based on the spectroscopical analysis of FT-IR and GC-MS, an isolated compound from *A. odoratissimus* was identified as triterpene, friedelinol. The compound has moderate cytotoxicity against a brine shrimp, *A. salina* with LC_{50} 48.39 ppm.

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