Isolation and Cytotoxic Assay of α-amyrin from *Callistemon citrinus* Stem Bark Against Cervical Cancer Cells (HeLa)

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Abstract. Cervical cancer attitudes a significant health challenge due to its high prevalence and mortality rates. As an alternative approach to treating cancer, scientists are exploring the use of bioactive compounds derived from herbal plants. One such plant is the bottle brush plant (*C. citrinus*). Isolation of α-amyrin from *C. citrinus* stem bark initiates by maceration using methanol and ethyl acetate. The phytochemical assay of *C. citrinus* extract contained alkaloids, terpenoids, tannins, and flavonoids. Antioxidant assay by DPPH method showed that the methanol and ethyl acetate extract had IC_{50} = 58.03, 84.21 ppm respectively. Fractionation of methanol extract was carried out by Vacuum Liquid Chromatography and purification by Column Chromatography. The isolated compound, namely isolate M-5b2, has a white needle crystal with melting point about 213.4-215.1°C. The structure of isolate M-5b2 was determined by spectroscopy analysis (UV-Vis, FT-IR, ^{1}H-NMR, ^{1}H↔^{1}H COSY, and GC-MS). The analysis of spectroscopic data from M-5b2 suggests that it is an α–amyrin compound. The cytotoxic activity of α–amyrin compounds against cervical cancer cells (HeLa) by MTT method exhibited low cytotoxic by IC_{50} value of 56.75 ppm.

Keywords: Cervical Cancer, *C. citrinus*, Cytotoxic Activity.

1 Introduction

Cancer is a condition where cells in the body tissues have abnormal growth and change into cancer cells [1]. Cancer is included in the category of diseases that cause death globally and can affect various organ tissues, one of which is the female reproductive organs such as the cervix [2]. Cervical cancer is from the cervix, a narrow opening into the uterus that connects to the vagina through the endocervical canal [3]. Cervical cancer has remained a health problem until now as seen from the high prevalence and mortality rates**.**

The Global Cancer Observatory claims that in 2020 Asian countries will have a major role in cancer cases globally. A total of 19,292,798 people worldwide have cancer and Asian countries have the largest population with 9,503,710 people diagnosed with

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cancer. The Southeast Asian region including Indonesia is the country with the first largest cancer patient with 396,914 cases and followed by Thailand as the second with 190,636 cases, this shows that cancer that occurs in Indonesia is quite high [4]. WHO data from the Global Cancer Observatory in 2020 also reported cases of cervical cancer in women in the world and in Indonesia. Cervical cancer ranks fourth highest in the world, with 341,831 cases of women are dead from a total of 604,127 cases of women diagnosed with cervical cancer. Indonesia ranks second highest with 21,003 cases of women died from a total of 36,633 cases of women diagnosed with cervical cancer [4].

Cervical cancer is usually caused by Human papillomavirus (HPV) infection and other risk factors include sexually transmitted diseases such as HIV and chlamydia trachomatis, smoking, high parity and prolonged use of oral contraceptives [5]. Cervical cancer requires special treatment, so various new drug developments and research are carried out as an effort to prevent, treat, and detect cervical cancer early [2]. Special treatment methods are needed for patients who have infected cervical cancer in order to reduce the liveliness of cervical cancer growth, which is currently known as chemotherapy [6].

Chemotherapy or cancer therapy with drugs has several limitations such as side effects, expensive, very complex and toxic. The mechanism of action of chemotherapy drugs is not selective because of its toxic nature so that it can cause normal cell division to be inhibited and drug resistance to occurs [9]. Side effects that can be caused by chemotherapy therapy include decreased blood production, hair loss, weakened immune system, heart disease, and nervous disorders. Another limitation is that cancer cells are resistant to cancer therapy drugs when mutated [7]. An alternative anticancer drug that is believed to be safe and has minimal side effects is the use of bioactive compounds from herbal plants [1]. Herbal plants produce bioactive compounds that are useful as drugs, one of which is the bottle brush plant (*Callistemon citrinus*). Based on the literature, *C. citrinus* plants can fight various diseases, one of which has the potential as an anticancer drug [11,12]. Analysis results from previous studies show that *C. citrinus* has the potential as a drug candidate to killed cancer cells [12].

The content of secondary metabolites in plants can be anticancer agent and lead to the development of new clinical drugs [8]. Research conducted by (Pendyala & Thakur, 2017) showed the content of secondary metabolite compounds in the methanol extract of *C. citrinus* stem bark consisting of alkaloids, flavonoids, steroids/terpenoids, saponins, and tannins [13]. Bioactive compounds in *C. citrinus* stem bark are an important factor in anticancer assay. Research that focuses on the use of *C. citrinus* stem bark as sample to be studied is still limited, therefore in this study samples of *C. citrinus* stem bark extracts were used to determine their potential as cervical anticancer against HeLa cells.

2 Materials and Method

2.1 Tools and Materials

The tools used in this study include glassware, 10 mL and 100 mL vials, stirring rods, vaporizer cups, dropper pipettes, measuring pipettes, a set of vacuum liquid chromatography (VLC) tools, a set of column chromatography (CC) tools, melting point measuring instrument, UV lamp (254 and 366 nm), capillary tube, rotary evaporator (Heildolph), UV-Vis spectrophotometer (Genesys 150), FT-IR spectrophotometer (Scimitar 2000), NMR spectrophotometer (Agilent 500 MHz), and GC-MS spectrophotometer.

The materials used in this study include bottle brush stem bark (*C. citrinus*) from the Sumatera Institute of Technology (ITERA) South Lampung. The chemicals used consisted of ethyl acetate ($C_4H_8O_2$), methanol (CH_3OH), n-hexane (n-C_6H_{14}), acetone (C_3H_6O), vanillin sulphate 1%, sulfuric acid (H_2SO_4), dichloromethane (CH_2Cl_2), silica gel G 60 (Merck) for impregnation, silica gel Merck 35-70 Mesh 60 (Merck) for VLC and CC, silica gel 60 F_{254} 0.25 mm TLC plate (Merck). Mayer's reagents, Dragendorff's reagents, Liebermann-Burchard reagents, $FeCl_3$ 1% solution, $MgCl_2$ solution, aquadest, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. Cancer cell culture media consisted of α-MEM (α Minimum Essential Medium), RPMI (Roswell Park Memorial Institute 1640), and D-MEM (Dulbecco's Modified Eagle's Medium). The dye used was Tryptone Blue (Merck). Trypsin and PBS were used for subculturing and harvesting cancer cell cultures.

2.2 Plants Determination

Samples of bottle brush plants (*C. citrinus*) obtained from the Sumatera Institute of Technology (ITERA) South Lampung with data on the coordinate point of bottle brush plant sampling, namely 5º21'38.4''LU 105º18'56.2''BT. The plant samples were made for specimens to be determined by comparing the plant samples with reference library data (storage). The plant determination test was carried out at Herbarium Bogoriense (BRIN) Cibinong, West Java and it was confirmed that the sample was a type of *Callistemon citrinus*.

2.3 Extraction and Isolation

Bottle brush stem bark that has become fine powder is weighed as much as 750 grams, then macerated with methanol and ethyl acetate solvents for 3 × 24 hours at room temperature. Then filter the macerated extract and the filtrate is vacuum evaporated with a rotary evaporator (50ºC/110 rpm) and obtained crude extract. Fractionation of methanol crude extract (22 grams) was carried out by VLC using a stationary phase in the form of silica gel Merck 35-70 Mesh 60 (Merck) and a mobile phase using solvents with increasing levels of polarity (n-hexane 100% to ethyl acetate 100%). The results of fractionation with VLC obtained 31 fractions then combined spot TLC with the same separation pattern into 13 fractions namely (M-1 to M-13).

Fraction M-5 (357.3 mg) was selected for further purification using CC with ethyl acetate : n-hexane (1:9) eluent to produce 4 fractions namely M-5a, M-5b, M-5c, and M-5d. Fraction M-5b (72.5 mg) was purified again with ethyl acetate : n-hexane (1:29) eluent to produce 3 fractions namely M-5b1, M-5b2, and M-5b3. Fraction M-5b2 labelled as isolate M-5b2 was tested for purity with three eluent test and melting point test. Isolate M-5b2 which is considered pure was weighed to produce (16 mg).

2.4 Phytochemical Assay

Samples were weighed as much as 1 gram and dissolved in 10 mL of solvent (methanol/ethyl acetate). Extracts as much as 0.5 mL were taken and put each extract into a test tube then added Mayer and Dragendorff reagents (Alkaloids), Liebermann-Burchard (Steroids and Terpenoids), $MgCl_2$ solution (Flavonoids), $FeCl_3$ 1% solution (Tannins), and aquadest (Saponins).

2.5 Antioxidant Assay

Quantitative antioxidant assay was conducted using the DPPH method. Standard solution and sample solution with a concentration of 1000 ppm as stock solution were made by dissolving 10 mg of vitamin C powder or sample extract with methanol in a 10 mL volumetric flask and the volume is sufficied up to the limit mark. Vitamin C standard solution and sample solution each with a concentration series (6.25 ppm; 12.5 ppm; 25 ppm; 50 ppm; 100 ppm) were serially diluted then added 0.1 mM DPPH solution as much as 2.9 mL quickly then incubated in a dark room for 30 minutes. Then, the absorbance was measured at (λ = 517 nm) using the UV-Vis spectrophotometer. Antioxidant activity against DPPH was analyzed through the calculation of percentage inhibition in equation (1) [10].

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \; x\; 100\% \qquad (1)$$

The IC_{50} value was calculated using its linear regression equation.

2.6 Structure Identification of Isolated Compounds

Structural identification of α-amyrin compounds was carried out using Ultra Violet-Visible (UV-Vis) spectroscopy, Fourier Transformed Infrared (FT-IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy for 1D (^{1}H-NMR) and 2D (^{1}H↔^{1}H COSY) and Gas Chromatography and Mass (GC-MS).

2.7 Cytotoxic Assay against HeLa Cells

Pure samples in the form of crystals that have been obtained as much as 2 mg are carried out cytotoxic assay with the MTT method. The cytotoxic assay against cervical cancer cells (HeLa) begins with harvesting cancer cells when 80-90% cell growth in petri dishes is met, then the number is calculated and cell dilution is carried out in RPMI 1640 culture medium. A total of 100 µL of cell suspension in RPMI 1640 medium was grown on 96-microplates with a concentration of 2×10^3 cells/well. Calculation of cell concentration using haemocytometer with Tryptone Blue cell dye. Cells

were incubated for 24 hours in a 5% CO_2 incubator at 37°C and added samples and then incubated again for 72 hours then MTT reagent was added as much as 100 µL/well and incubated for 3 hours in dark conditions. Furthermore, the addition of DMSO 100 µL/well to dissolve the formazan crystals and analyze the absorbance read with a microplate reader at 595 nm [14,15].

3 Results and Discussion

3.1 Extraction and Phytochemical Assay

The crude methanol and ethyl acetate extracts were obtained as much as 28.9624 grams and 9.8897 grams. Phytochemical assay results showed that methanol and ethyl acetate extracts of *C. citrinus* stem bark were positive for alkaloids, terpenoids, flavonoids, and tannins, while saponins were only found in methanol extracts (see Fig. 1 and Fig. 2).

Fig. 1. Phytochemical assay of methanol extracts.

Fig. 2. Phytochemical assay of ethyl acetate extracts.

3.2 Antioxidant Assay

The antioxidant assay of *C. citrinus* stem bark, vitamin C was used as a comparison solution and sample solution were methanol and ethyl acetate crude extracts. Determination of antioxidant activity with DPPH method used IC_{50} parameter which is the concentration of the sample required in capturing DPPH radicals as much as 50% [16]. The following is a graph between concentration and % inhibition to determine IC_{50} (see Fig. 3 and Fig. 4).

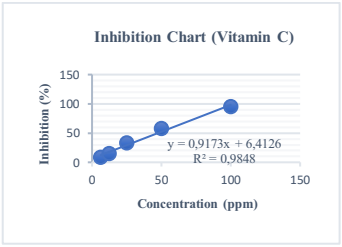

Fig. 3. Graph of % inhibition vs concentration of vitamin C.

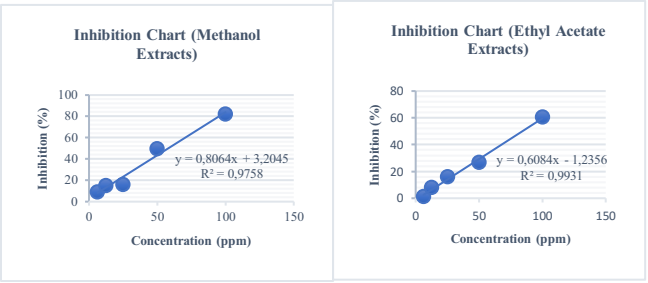

Fig. 4. Graph of % inhibition vs concentration of methanol and ethyl acetate extracts.

The results of antioxidant assay on the comparison solution (vitamin C) and *C. citrinus* stem bark extracts as shown in Table 1.

Table 1. Antioxidant activity of vitamin C and *C. citrinus* stem bark extracts.

Sample	IC_{50} value (ppm)	Antioxidant Activity
Vitamin C	47.51	Very strong
Methanol extract	58.03	Strong
Ethyl acetate extract	84.21	Strong

3.3 Isolation of Isolated Compounds

Fractionation using VLC was carried out on methanol extracts, this is because methanol extracts have a lot of extract mass, have more secondary metabolite content and strong antioxidant activity. The results of purification of isolated compounds, namely isolate M-5b2 as much as 16 mg of white needle crystal with the melting point of 213.4ºC-215.1ºC (see Fig. 5).

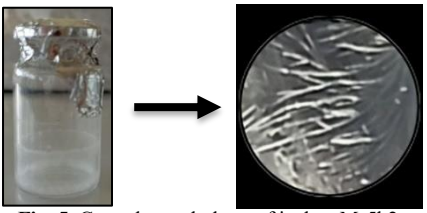

Fig. 5. Crystal morphology of isolate M-5b2.

3.4 Phytochemical Assay of Isolated Compounds

Identification of isolate M-5b2 was then carried out with phytochemical assay with several reagents (Fig. 6) and TLC assay to determine the type of compound class of isolate M-5b2 (Fig. 7). The results of the phytochemical assay showed that isolate M-5b2 gave a positive result against Liebermann-Burchard reagent characterised by the formation of a purple spot after proven by TLC test so that it can be indicated that isolate M-5b2 belongs to the group of terpenoid compounds.

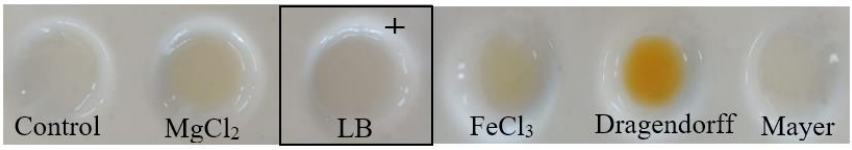

Fig. 6. Phytochemical assay of isolate M-5b2 with several reagents.

Fig. 7. TLC test of isolate M-5b2 with Libermann-Burchard reagent.

3.5 Structure Identification of Isolated Compounds

Analysis of the structure of isolate M-5b2 with UV-Vis spectra shows absorption at λmax = 284 nm indicating the excitation of electrons from the π→π* orbital. The π→π* electron transition occurs because there is a chromophore, an atomic group that can absorb light at a certain wavelength. The π→π* electron transition identifies that isolated M-5b2 has a typical chromophore group in the form of an unconjugated double bond (C=C) (see Fig. 8).

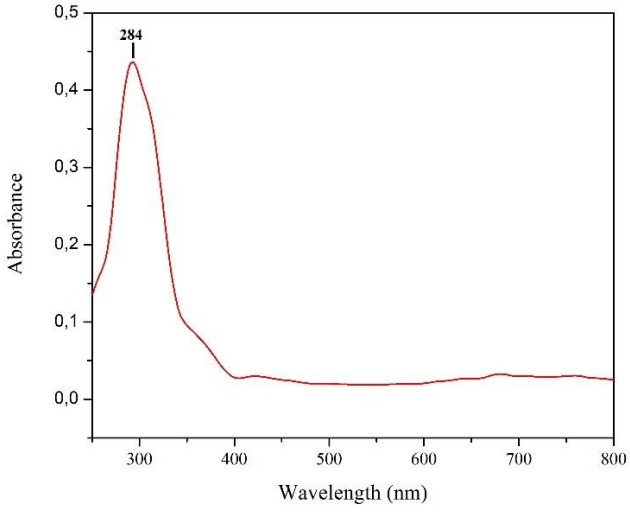

Fig. 8. UV-Vis spectrum of isolate M-5b2.

The FT-IR spectra results (see Fig. 9 and Table 2).

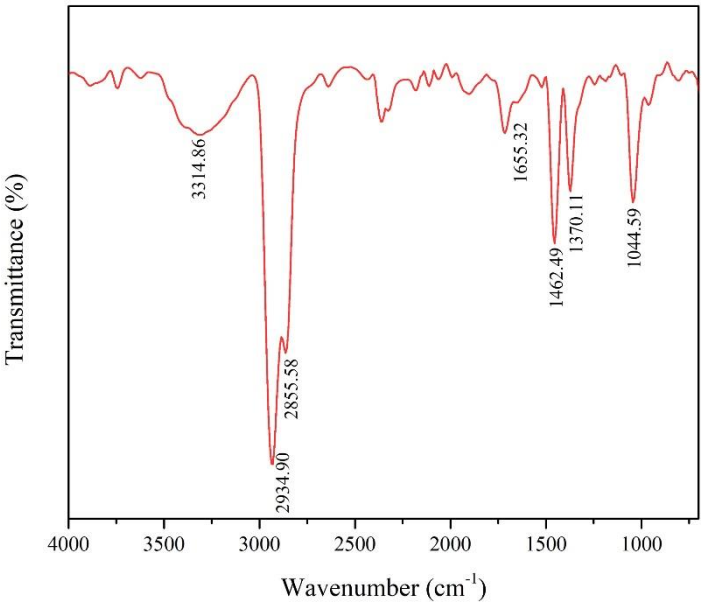

Fig. 9. FT-IR spectrum of isolated M-5b2.

Table 2. Interpretation of FT-IR spectrum data of isolated M-5b2.

Wavenumber (cm^{-1})	Function Group
3314.86	O-H stretching
2934.90	C-H (sp^3) stretching
2855.58	C-H (sp^3) stretching
1655.32	C=C stretching
1462.49	C-H (CH_2) bending
1370.11	C-H (CH_3) bending
1044.59	C-OH bending

The results of the FT-IR spectrum exhibits a broad absorption in the wavenumber region of 3314.86 cm^{-1} the existence of stretching vibrations from hydroxyl groups (-OH) bound to atoms carbon which is strengthened by the existence of secondary alcohol (-CH-OH) absorption at wavenumber 1044.59 cm^{-1} which shows bending vibrations of -C-O bonds [17]. Absorption with wavenumber in the region of 2934.90 cm^{-1} and 2855.58 cm^{-1} exhibits the existence C-H aliphatic stretching vibrations of -CH_2 and -CH_3 groups which indicate the structure of the isolate compound M-5b2 contained methyl and methylene groups supported by the C-H aliphatic bending vibrations in the wavenumber region 1462.49 cm^{-1} for the -CH_2 (methylene) group and

1370.11 cm^{-1} for the -CH_3 (methyl) group which indicates the existence geminal dimethyl group -$CH(CH_3)_2$ that is two methyl groups bonded to the same carbon atom. The absorption band at wavenumber region of 1655.32 cm^{-1} indicates the existence stretching vibration of one carbon double bond (-C=C) in the structure [18].

The ^{1}H-NMR spectrum of isolate M-5b2 (see Fig. 10) exhibits the typical characteristics of the ^{1}H-NMR signal pattern for triterpenoid compounds, seen in the chemical shift region (δ_H) < two ppm showing proton signals that overlaps on aliphatic protons (CH_3, CH_2, and CH), which is an protons bound to the cyclic ring of the basic framework of triterpenoids that are not well separated [19]. The ^{1}H-NMR spectrum of isolate M-5b2 exhibits the existence of fifty protons containing eight methyl (CH_3), nine methylene (CH_2), seven methines (CH) and one hydroxy group (OH). The signal in the chemical shift region (δ_H) 0.74-5.33 ppm indicates isolated M-5b2 does not have an aromatic structure, but has a proton (H) on the cyclic carbon.

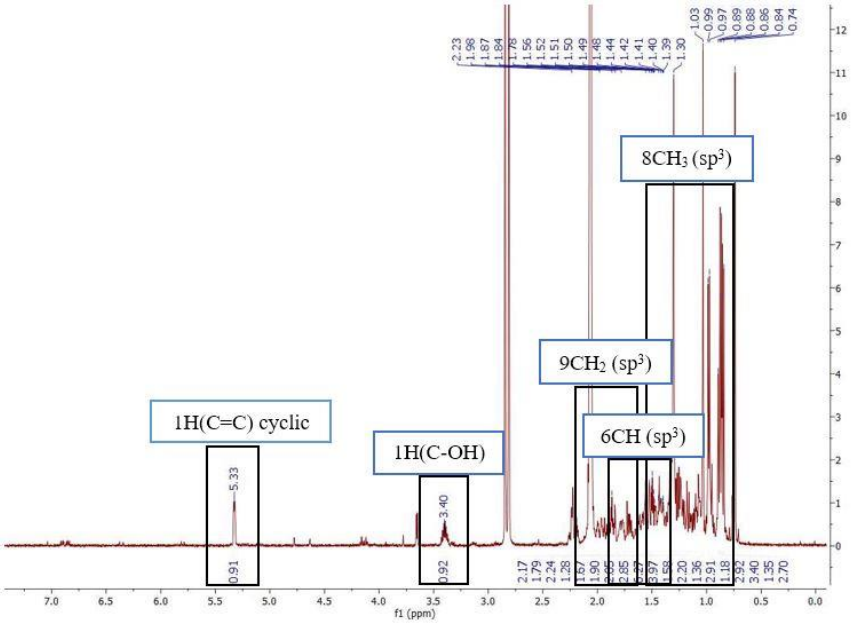

Fig. 10. ^{1}H-NMR spectrum of isolate M-5b2.

The data from the ^{1}H-NMR spectrum of isolate M-5b2 shows that there are eight methyl proton signals with 3H integration indicating the existence of methyl protons, namely the existence of two -CH_3 groups in the geminal dimethyl position. The signal in the chemical shift region (δ_H) 3.40 ppm is a typical signal of methine protons binding to the C atom to which the hydroxyl group (OH) is bound in the C-3 position. The signal in the region of (δ_H) 5.33 ppm indicates the existence of proton bound to (C=C), which is an olefinic proton substituted on the cyclic ring at positions C-12 and C-13. The following is the interpretation of the spectrum data ^{1}H-NMR isolate M-5b2 as in Table 3.

Table 3. Interpretation of ^{1}H-NMR spectrum data of isolated M-5b2.

Position proton (H)	C/H	δ_H Isolate M-5b2	δ_H Literature [20]
H-1	CH_2	1.76 (m)	1.68 (m)
H-2	CH_2	1.98 (m)	2.03 (m)
H-3	CH	3.40 (m)	3.22 (dd)
H-4	Cq	-	-
H-5	CH	1.06 (t)	0.74 (d)
H-6	CH_2	1.54 (m)	1.57 (m)
H-7	CH_2	1.40 (m)	1.36 (m)
H-8	Cq	-	-
H-9	CH	1.50 (m)	1.54 (m)
H-10	Cq	-	-
H-11	CH_2	2.23 (m)	1.91 (m)
H-12	CH	5.33 (t)	5.12 (t)
H-13	C=C	-	-
H-14	Cq	-	-
H-15	CH_2	1.88 (m)	1.61 (t)
H-16	CH_2	1.84 (m)	1.83 (t)
H-17	Cq	-	-
H-18	CH	1.45 (m)	1.31 (s)
H-19	CH	1.48 (m)	1.36 (m)
H-20	CH	1.04 (m)	0.87 (m)
H-21	CH_2	1.40 (m)	1.39 (m)
H-22	CH_2	1.54 (m)	1.41 (t)
H-23	CH_3	0.84; 0.86; 0.89 (s)	0.99 (s)
H-24	CH_3	0.74 (s)	0.95 (s)
H-25	CH_3	0.86 (s)	0.96 (s)
H-26	CH_3	1.28 (s)	1.01 (s)
H-27	CH_3	1.28 (s)	1.07 (s)
H-28	CH_3	1.01 (s)	0.80 (s)
H-29	CH_3	0.88 (d)	0.79 (d)
H-30	CH_3	0.97 (d)	0.91 (d)

The structural analysis of isolate M-5b2 was strengthened by using 2D NMR spectrum (^{1}H↔^{1}H COSY). The following is shown the spectrum of ^{1}H↔^{1}H COSY isolate M-5b2 (see Fig. 11).

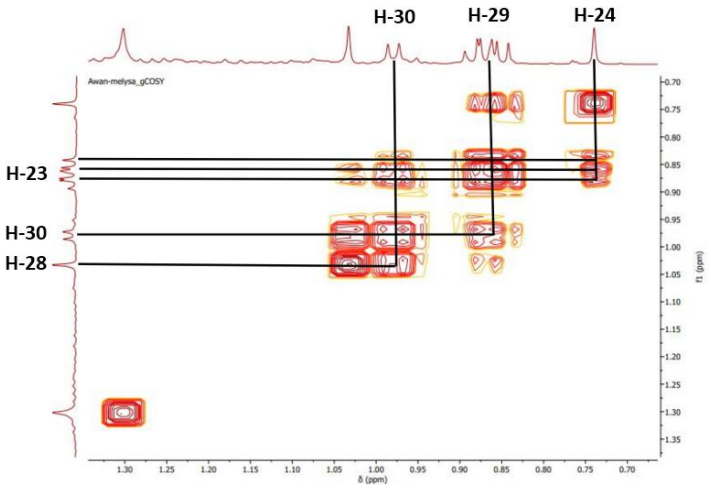

Fig. 11. $^{1}H \leftrightarrow {}^{1}H$ COSY spectrum of isolate M-5b2.

The results of 2D NMR spectrum analysis ($^{1}H \leftrightarrow {}^{1}H$ COSY) show the correlation of neighbouring protons, which is the proton signal at (H-23) correlates with the proton signal at (H-24). The proton signal at (H-29) correlates with the proton signal at (H-30). The proton signal at (H-28) correlates with the proton signal at (H-30).

Structure elucidation analysis of isolate M-5b2 by gas chromatography-mass spectroscopy (GC-MS). Spectral readings were carried out with the two methods that are GC and MS analysis. The following GC chromatogram results isolate M-5b2 (see Fig. 12).

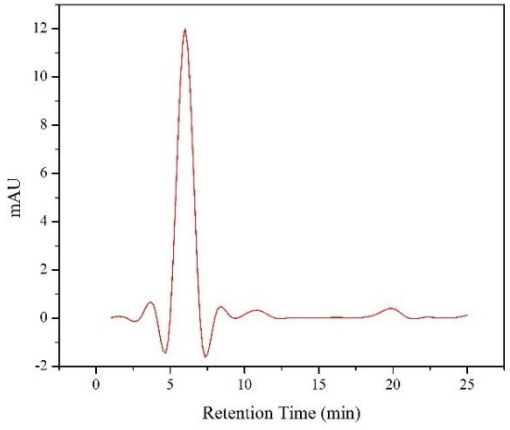

Fig 12. GC chromatogram of isolate M-5b2.

Mass spectroscopy (MS) is carried out to identify the molecular weight and determination of the molecular formula of a compound. The following is the result of the MS spectrum of isolate M-5b2 (see Fig. 13).

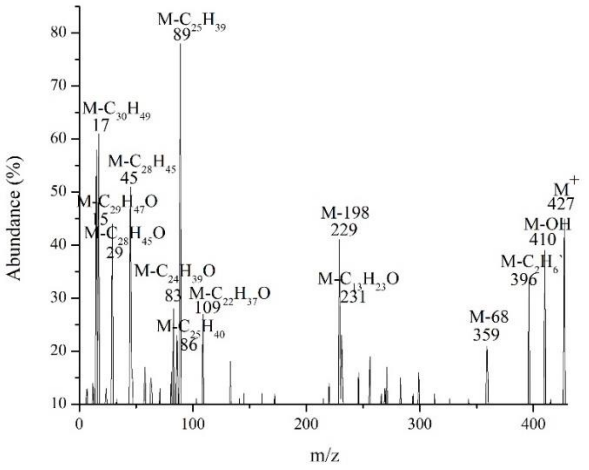

Fig. 13. GC-MS spectrum of isolate M-5b2.

The GC-MS spectra of isolate M-5b2 (see Fig. 12 and Fig. 13) show a major peak with a large peak height on the GC chromatogram when the retention time of 6 minutes has a high signal intensity.

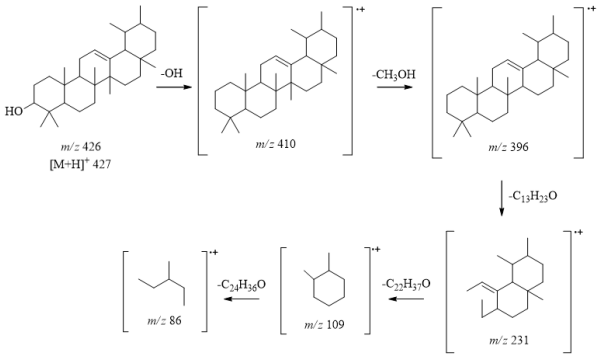

Fig. 14. Fragmentation pattern of MS spectrum of isolate M-5b2.

The MS spectrum shows the molecular ion mass at the peak *m/z* 427 indicating a compound with the molecular formula $C_{30}H_{50}O$ with the formula $[M+H]^+$ which is $[426+1]^+ = 427$. The fragmentation pattern of the MS spectrum of isolate M-5b2 (see Fig. 14) shows the peak at *m/z* 410 fragmented with the loss of hydroxy group (OH)• corresponds to (M-17). The peak at *m/z* 396 or the loss of (CH_3OH)• corresponds to (M-31). The peak at *m/z* 231 appears due to the loss of $(C_{13}H_{23}O)$• corresponds to (M-

196). The peak at *m/z* 109 appears due to the loss of $(C_{22}H_{37}O)$• corresponds to (M-318). The peak at *m/z* 86 appears due to the loss of $(C_{24}H_{36}O)$• corresponds to (M-341).

The results of spectroscopic analysis and comparison with literature compounds, the isolate M-5b2 is suggested to be a pentacyclic triterpenoid compound with a ursan basic skeleton, namely the α-amyrin compound with the molecular formula $(C_{30}H_{50}O)$ (see Fig. 15).

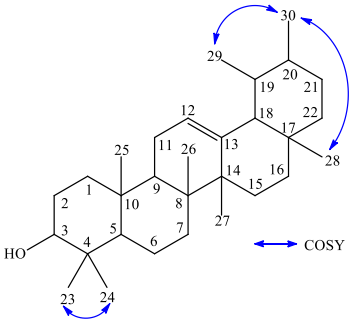

Fig. 15. Structure of the compound α–amyrin.

3.6 Cytotoxic Assay against HeLa Cells

Cytotoxic assay was conducted to determine the toxicity of isolated M-5b2 against HeLa cells. The MTT method is used to detect the presence of cell activity (proliferation) based on the ability of MTT (Methylthiazol Tetrazolium) is yellow colored form purple formazan by the enzyme succinate dehydrogenase (see Fig. 16).

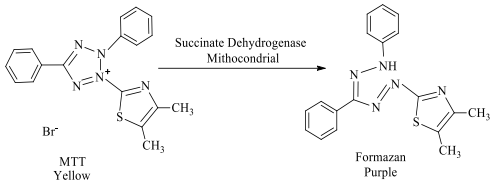

Fig. 16. MTT reduction reaction to form formazan [21].

The results of the cytotoxicity assay of isolate M-5b2 against HeLa cells are shown in Table 4.

Table 4. Cytotoxicity assay of isolate M-5b2 against HeLa cells.

Concentration (ppm)	% PP			% average PP	Standard Deviation (SD)
	Repeat 1	Repeat 2	Repeat 3		
3.125	89.6381	90.7393	90.5406	90.3060	0.5869
6.25	87.0261	87.8274	-	87.3630	0.4154
12.5	73.8462	74.2474	74.7487	74.2808	0.4521
25	-	59.9676	59.7866	59.6402	0.4202
50	-	52.3310	50.6000	51.1536	1.0202
100	41.8578	42.6590	43.6603	42.7257	0.9031

The results of cytotoxic assay data in Table 4 show the relationship between sample concentration and % inhibition of proliferation. The following profile of the relatioship between the concentration of the sample (isolate M-5b2) and the percentage of inhibition of HeLa cell proliferation after treatment (see Fig. 17).

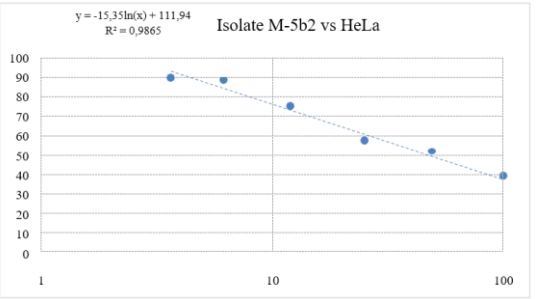

Fig. 17. Graph of the relationship between sample concentration (isolate M-5b2) and percentage inhibition of HeLa cell proliferation.

The toxicity parameter of a compound is determined by the IC_{50} value which is the 50% concentration of the sample required to inhibit cell proliferation. The American National Cancer Institute (NCI) states that the category of toxicity of a compound against cancer cells has an IC_{50} value which is a very active category ≤ 20 ppm, moderately active (moderate) 21-200 ppm, weak category 201-500 ppm, and non-toxic category > 500 ppm. The category of IC_{50} value by NCI also illustrates that if the IC_{50} value is smaller, the compound will be more toxic and vice versa [22]. The results of the MTT assay analysis show that isolate M-5b2 has an IC_{50} value of 56.75 ppm which indicates that in this concentration it can inhibit cell proliferation by 50% so that it is included in the moderately active category against HeLa cervical cancer cells with low cytotoxicity activity.

4 Conclusion

The results of spectroscopic data analysis of isolated compounds from bottle brush stem bark extract (*C. citrinus*) obtained from Institut Teknologi Sumatera (ITERA) South Lampung are suggested to be pentacyclic triterpenoid compounds, namely α-amyrin compounds. The cytotoxic activity of α–amyrin compounds against cervical cancer cells (HeLa) by MTT method exhibited low cytotoxic.

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References

1. R. M. Zafrial and R. Amalia, "Artikel Tinjauan : Anti Kanker Dari Tanaman Herbal," *Farmaka,* vol. 16, no. 1, pp. 15-23, 2018
2. M. Triharini, E. Yunitasari, N. K. A. Armini, T. Kusumaningrum, R. Pradanie, and A. A. Nastiti, "Pemberdayaan Perempuan Melakukan Deteksi Dini Kanker Serviks Melakukan Pelatihan Metode Reproductive Organ Self Examination (Rose) Sebagai Upaya Deteksi Dini Penyakit Kanker Serviks," *Jurnal Pengabdian Masyarakat Dalam Kesehatan,* vol. 1, no. 1, pp. 14-20, 2019.
3. C. A. Burmeister et al., "Cervical cancer therapies: Current challenges and future perspectives," *Tumour Virus Research*, vol. 13. Elsevier B.V., Jun. 01, 2022. doi: 10.1016/j.tvr.2022.200238.
4. GLOBOCAN, "Cancer Today," International Agency for Research on Cancer, 2020. https://gco.iarc.fr/.
5. M. R. Mwantake, H. D. Kajoka, F. C. Kimondo, C. Amour, and I. B. Mboya, "Factors associated with cervical cancer screening among women living with HIV in the Kilimanjaro region, northern Tanzania: A cross-sectional study," *Prev Med Rep*, vol. 30, p. 101985, Dec. 2022, doi: 10.1016/j.pmedr.2022.101985.
6. L. Changxing et al., "Biotechnological approaches to the production of plant-derived promising anticancer agents: An update and overview," *Biomedicine and Pharmacotherapy*, vol. 132. Elsevier Masson s.r.l., Dec. 01, 2020. doi: 10.1016/j.biopha.2020.110918.
7. J. Iqbal et al., "Plant-derived anticancer agents: A green anticancer approach," *Asian Pacific Journal of Tropical Biomedicine*, vol. 7, no. 12. Hainan Medical University, pp. 1129–1150, Dec. 01, 2017. doi: 10.1016/j.apjtb.2017.10.016.
8. A. M. L. Seca and D. C. G. A. Pinto, "Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application," *International Journal of Molecular Sciences*, vol. 19, no. 1. MDPI AG, Jan. 16, 2018. doi: 10.3390/ijms19010263.
9. F. Malik et al., "Aktivitas Sitotoksik Ekstrak Etanol Bunga Kasumba Turate (*Carthamus tinctorius* Linn.) Terhadap Lini Sel Kanker Payudara T47D," *Jurnal Farmasi Sains dan Praktis*, vol. 7, no. 3, pp. 384–392, Dec. 2021.
10. L. Y. Leng, Nadzrin, A. R. Shaari, A. R. Norawanis, and C. Y. Khor, "Antioxidant capacity and total phenolic content of fresh, oven-dried and stir-fried tamarind leaves," *Current Research in Nutrition and Food Science*, vol. 5, no. 3, pp. 282–287, Dec. 2017, doi: 10.12944/CRNFSJ.5.3.13.
11. H. Renggana, Y. E. Hadisaputri, and A. Subarnas, "Myrtaceae Anticancer Activities," *Jurnal Ilmiah Farmako Bahari,* vol. 9, no. 2, pp. 23-32, 2018.
12. D. M. Nyaga, K. P. Kamweru, and K. N. Uttam, "Bioprospecting of *Callistemon citrinus* explored for present chemical species using FTIR," *~ 90 ~ The Pharma Innovation Journal*, vol. 8, no. 10, pp. 90–95, 2019, [Online]. Available: http://www.thepharmajournal.com.
13. V. Pendyala and S. Thaakur, "Phytochemical and pharmacological evaluation of *Callistemon citrinus* for antidepressant activity in albino mice," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 10, no. 8, pp. 232–234, 2017, doi: 10.22159/ajpcr.2017.v10i8.18998.
14. H. Amir and B. G. Murcitro, "Uji Microtetrazolium (MTT) Ekstrak Metanol Daun *Phaleria macrocarpa* (Scheff.) Boerl Terhadap Sel Kanker Payudara MCF7," *Jurnal Pendidikan dan Ilmu Kimia*, vol. 1, no. 1, pp. 27–32, 2017.

15. M. Maryati and E. Sutrisna, "Fraksi Petroleum Eter Tanaman Ceplukan (*Physalis angulata* L.) Menghambat Proliferasi dan Memacu Apoptosis pada 69 Sel HeLa," Biota J. Ilm. Ilmu-Ilmu Hayati, vol. 16, no. 1, pp. 80–87, 2011, doi: 10.24002/biota.v16i1.62.
16. F. Setiawan, O. Yunita, and A. Kurniawan, "Uji aktivitas antioksidan ekstrak etanol kayu secang dan FRAP," Media Pharm. Indones., vol. 2, no. 2, pp. 82– 89, 2018.
17. R. Kurniawan, T. Suhartati, Y. AS, D. Meriyanti, and S. Sukrasno, "Potential Antibacterial Activity of Bioactive β-sitosterol from Root Bark of *Rhizophora apiculata* from Lampung Coastal," *Jurnal Kimia Sains dan Aplikasi*, vol. 24, no. 4, pp. 114–119, Apr. 2021, doi: 10.14710/jksa.24.4.114-119.
18. M. Latief et al., "Isolasi Senyawa Triterpenoid Ekstrak Etanol Daun Jeruju (*Achantus ilicifolius*) dan Aktivitas Antibakterinya Terhadap *S. aureus* dan *E. coli*," *Jurnal Kimia*, vol. 16, no. 1, pp. 34–44, Jan. 2022.
19. Rugayah, Rudiyansyah, and A. Jayuska, "Karakterisasi Senyawa Triterpenoid Dari Daun Jabon (*Anthocephalus cadamba* (Roxb.) Miq)," *JKK*, vol. 6, no. 2, pp. 56–63, 2017.
20. N., N. H. Soekamto, A. Ahmad, and. S., "α–Amyrin and β-Sitosterol From Bark Extract Of *Rhizophora Mucronata* Lamk. and Their Cytotoxic Activities Against HeLa Cell Line," *International Research Journal Of Pharmacy*, vol. 9, no. 9, pp. 75–79, Oct. 2018, doi: 10.7897/2230-8407.099191.
21. T. Mavromoustakos, A. G. Tzakos, and S. Durdagi, "*Supramolecules in Drug Discovery and Drug Delivery: Methods and Protocols,"* 1st ed., vol. 2207. Humana, 2020.
22. Adisty Ridha Damasuri, Eti Nurwening Sholikhah, and Mustofa, "Cytotoxicity of ((E)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) on HeLa cell line," *Indonesian Journal of Pharmacology and Therapy*, vol. 1, no. 2, Dec. 2020, doi: 10.22146/ijpther.606.

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