The Anticancer Activity of Phytoconstituents of the Stem of *Bouea macrophylla*

Tarso Rudiana*1*, Nani Suryani1, Dimas D. Indriatmoko2, Yusransyah Yusransyah3, Muhammad A. Hardiyanto4, Ricky Yohanis2, Siti Nurbayti4, Eka Nurdiansyah2, Hidayatul Fajri1, Noviany Noviyan5 and Sutopo Hadi5

1Department of Chemistry Faculty of Science, Pharmacy and Health, Universitas Mathla’ul Anwar, Jalan Raya Labuan-Pandeglang KM 23, Pandeglang Banten 42273, Indonesia.
2Department of Pharmacy Faculty of Science, Pharmacy and Health, Universitas Mathla’ul Anwar, Jalan Raya Labuan-Pandeglang KM 23, Pandeglang Banten 42273, Indonesia.
3Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Salsabila, Jalan Raya Serang-Pandeglang KM 06 No. 33 Serang Banten 42211, Indonesia.
4Department of Chemistry Faculty of Science and Technology, Universitas Islam Negeri (UIN) Syarif Hidayatullah Jakarta, Jalan Ir. H. Juanda No. 95, South Tangerang Banten 15412, Indonesia.
5Department of Chemistry Faculty of Mathematical and Natural Sciences Universitas Lampung, Bandar Lampung, Lampung 35141, Indonesia.

*Corresponding Author E-mail: tarso.rudiana@gmail.com
https://dx.doi.org/10.13005/bpj/2293
(Received: 12 October 2021; accepted: 08 December 2021)

Gandaria (*Bouea macrophylla* Griff) is a typical Asian plant that is commonly found in Indonesia with various secondary metabolite compounds such as phenolic, flavonoid and terpenoid. The purpose of this study was to isolate secondary metabolites from the stem extract of *B. macrophylla* and determine their activity against cancer cells MCF-7, A549, MDA-MB 231 and HCC-1954. The isolation of the compounds was conducted using various chromatographic techniques, the determination of the chemical structure of the isolates was performed using physicochemical methods including mass spectrometer and nuclear magnetic resonance, the determination of anticancer activity was carried out using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) i.e. MCF-7 and A549 cell lines; and dimetiltiazol-2-il) -2,5-diphenyltetrazolium bromide (MTT) for MDA-MB 231 and HCC-1954 cell lines. Four compounds namely stigmasterol (1), fustin (2), garbanzol (3) and methyl galat (4) were successfully isolated from the stem extract of *B. macrophylla*, which was obtained from Serang Regency, Indonesia. These compounds were then tested their anticancer activity against the cancer cells of Michigan Cancer Foundation-7 (MCF-7), human alveolar epithelial cells (A549), human breast cancer cell line-1954 (HCC-1954) and M.D. Anderson-Metastatic Breast-231 (MDA-MB-231). The results of anticancer test indicated that based on the IC50 values for all compounds tested, the compounds 2 and 4 were more active on HCC-1954 cell with IC50 values of 134.35 ± 44.62 and 153.69 ± 12.54 µg/mL, respectively, while the compound 3 was found to be the most active against MDA-MB-231 cell line with IC50 value of 233.41 ± 91.57 µg/mL.

**Keywords:** Anticancer, *B. Macrophylla*, Cytotoxicity, Phytoconstituents.

---

The *B. macrophylla* (Anacardiaceae) is a high fruit-producing plant rich in antioxidant compounds. It is common in Indonesia, especially on the islands of Sumatra, Java, Kalimantan and Maluku.1,2 The methanol extract of *B. macrophylla* fruit has antioxidant activity with an IC50 value of...
16.29 mg/mL. The methanol extract and the fruit skin as well as the fruits of *B. macrophylla* has been reported to be active as antioxidant. The fruit of *B. macrophylla* contains compounds of flavonoid class with an antioxidant activity value of IC$_{50}$ 2.43 ìg/mL, using in vitro 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity method. The antioxidant activity has an important correlation with anticancer activity. Kulsum *et al.* (2018) reported that the activity of antioxidants is proportionally correlated with anticancer activity in the test of amla and ginger extract with a probability value under 0.05. Their results indicated that compounds in the extract with an excellent antioxidant activity also have good anticancer activity. Gandaria has been reported to show a good antioxidant activity; thus it makes gandaria has possibility to show a good anticancer activity, too.

Andina and Musfirah reported that the ethanol extract of *B. macrophylla* leaves has a strong antioxidant activity with an IC$_{50}$ value of 55.83 ìg/mL. They also demonstrated that the antioxidant activity of the ethanol extract of the stem bark of *B. macrophylla* with an IC$_{50}$ value of 20.03 ìg/mL is greater than that of the leaf ethanol extract with an IC$_{50}$ value of 55.83 mg/mL. According to Rudiana *et al.* (2018), the ethyl acetate extract from gandaria stems (*B. macrophylla*) has the best antioxidant activity compared to n-hexane and methanol extracts with an IC$_{50}$ value of 4.89 ìg/mL.

The seed extract of *B. macrophylla* has been reported to have anticancer activity against human hypopharyngeal FaDu (HTB-43), MCF-7 and MDA-MB-231 cells with IC$_{50}$ values of 34.36; 59.07; 28.65 ìg/mL, respectively. The seed extract of *B. macrophylla* contains pentagalloyl glucose and ethyl gallate compounds, which can inhibit MCF-7 cells through the apoptotic pathway. Besides that, the seed extract of *B. macrophylla* can inhibit the growth of leukemia and lung cancer cells with IC$_{50}$ values ranging from 3 to 45 ìg/mL.

The exploration of pure phytoconstituents isolation of the stem of *B. macrophylla* has not been investigated. In our previous work, two compounds, luteolin and naringenin, have been identified in the ethyl acetate extract of *B. macrophylla* stem using liquid chromatography-mass spectrometry. Still, they were not isolated. The previous works on *B. macrophylla* mostly focused on the chemical content of their extracts. The present work aims to study the isolation of of secondary metabolites in the stem of *B. macrophylla* and determined the anticancer activity of the compounds isolated against MCF-7, HCC-1954, MDA-MB-231, and A549 cell lines.

**MATERIALS AND METHODS**

**Plant Material**

The stem of *B. macrophylla* was obtained from Serang District, Banten Province of Indonesia and identified at the Herbarium Bogoriense, a Center for Biological Research, Indonesian Institute of Research, Cibinong with voucher specimen number of 1068/IPH.1.01./If.07/VI/2018.

**General Experiment**

Thin layer chromatography analysis was carried out using silica gel on an aluminum layer (Merck Kieselgel 60 F254), monitoring TLC under UV lamps 254 and 365 nm. The vacuum liquid chromatography was performed using silica gel 60 G (Merck) as the stationary phase and silica gel 60 (Merck) in chromatography gravity column. The chemical structure of the isolates was determined using spectroscopic techniques including mass spectroscopy (Waters UPLC-MS/MS H-Class TQD), and 1H- and 13C-NMR spectroscopy which were obtained with JEOL ECA 500 with frequencies at 500 MHz and 125 MHz, respectively.

**Extraction and Isolation**

The stem powder of *B. macrophylla* (7.6 kg) was macerated in stages with n-hexane, ethyl acetate, and methanol (Technical, Pha Che, Indonesia) for 3 x 24 hours each using similar procedure available in the literatures. Each extract was tested anticancer activity against MCF-7, HCC 1954, MDA-MB 231, and A549 cell lines. The n-hexane extract (21 g) was separated by VLC using the mobile phase n-hexane: ethyl acetate: methanol: acetone in a 10% polarity gradient in such a way that the A-B fraction was obtained. Fraction A was purified by CC using n-hexane, ethyl acetate as the stationary phase to produce compound I (28 mg).

The ethyl acetate extract (19.90 g) was separated by VLC using methylene chloride: ethyl...
acetate: ethanol as the mobile phase to produce the A-K fraction. The G fraction (594.70 mg) was purified by CC using n-hexane: MTC: ethyl acetate as the mobile phase to give compound 3 (4.7 mg). Fraction J (7 g) was purified by CC using n-hexane: ethyl acetate: methanol in 10% gradient as the mobile phase to obtain the A-J fraction. The G fraction (778.2 mg) was purified by CC using n-hexane, and the absorbance of the reaction was measured using an ELISA reader at a wavelength of 550 nm.18

RESULTS AND DISCUSSION

The Isolated Compounds

Four compounds were successfully isolated. The separation is guided by spot pattern. The compounds were as follows: Stigmasterol (1) (Figure 1): colourless crystal, \(^{1}H\)-NMR (in CDCl\(_3\), 500 MHz) \(\delta_H (ppm): 6.40 \text{ (C-1); 36.3 (C-2); 71.9 (C-3); 42.3 (C-4); 140.9 (C-5); 120.7 (C-6); 32.0 (C-7); 32.0 (C-8); 50.3 (C-9); 36.5 (C-10); 21.4 (C-11); 39.2 (C-12); 42.4 (C-13); 56.9 (C-14); 24.5 (C-15); 29.2 (C-16); 56.2 (C-17); 12.2 (C-18); 19.5 (C-19); 39.9 (C-20); 23.2 (C-21); 138.5 (C-22); 129.4 (C-23); 51.4 (C-24); 31.8 (C-25); 20.0 (C-26); 21.2 (C-27); 25.6 (C-28) and 12.2 (C-29). UPLC-QTOFMS m/z 411.24 [M-] (calculated (calcd.) for C\(_{29}\)H\(_{48}\)O, m/z 412.69).

Fustin (2) (Figure 1): yellow amorphous, \(^{1}H\)-NMR (in acetone-d\(_6\), 500 MHz) \(\delta_H (ppm): 6.40 \text{ (H, d, J = 2.5 Hz, H-8); 6.62 (1H, dd, J = 8.5 and 2 Hz, H-6); 6.86 (1H, dd, J = 8 and 2.5 Hz, H-5'); 6.92 (1H, td, J = 8 and 2 Hz, H-6'); 7.07 (1H, d, J = 2.5 Hz, H-2'); 7.17 (1H, d, J = 8.5 Hz, H-5); 4.98 (1H, d, J = 12 Hz, H-2) and 4.53 (1H, d, J = 11.5 Hz, H-3).}^{13}C-NMR (in acetone-d\(_6\), 125 MHz): \(\delta_C (ppm): 74.0 (C-3); 85.0 (C-2); 103.1 (C-8); 111.8 (C-6); 115.8 (C-2'); 113.1 (C-10); 115.9 (C-5'); 120.9 (C-6'); 129.8 (C-5); 130.1 (C-1'); 145.8 (C-1); 140.9 (C-4); 132.5 (C-2); 71.9 (C-3); 42.3 (C-4); 140.9 (C-5); 120.7 (C-6); 32.0 (C-7); 32.0 (C-8); 50.3 (C-9); 36.5 (C-10); 21.4 (C-11); 39.2 (C-12); 42.4 (C-13); 56.9 (C-14); 24.5 (C-15); 29.2 (C-16); 56.2 (C-17); 12.2 (C-18); 19.5 (C-19); 39.9 (C-20); 23.2 (C-21); 138.5 (C-22); 129.4 (C-23); 51.4 (C-24); 31.8 (C-25); 20.0 (C-26); 21.2 (C-27); 25.6 (C-28) and 12.2 (C-29). UPLC-QTOFMS m/z 411.24 [M-] (calculated (calcd.) for C\(_{29}\)H\(_{48}\)O, m/z 412.69).

The anticancer activity against HCC 1995 and MDA-MB 231 cell lines was analyzed using the MTS assay method which were carried out at The Biological Activity Laboratory, the Central Laboratory, Universitas Padjadjaran, Bandung, Indonesia: the cells were cultured on RPMI media (Sigma-Aldrich) containing 10% FBS and antibiotics, and trypsin-EDTA was added and incubated for 2 hours. The MTT reaction was stopped using diphenyltetrazolium bromide (MTT) compound and incubated again for 24 hours, and 3-(4,5-Dimetiltiazol-2-il)-2,5-diphenyltetrazolium bromide (MTT) compound was added to each well containing 15,000 cells and incubated for 2 hours. The MITT reaction was measured using an ELISA reader at a wavelength of 550 nm.18

The anticancer activity of MCF-7 breast adenocarcinoma (ATCC HTB-22) and A549 Lung Carcinoma (ATCC CCL-185) cell lines were analysed using the MTS assay method which were carried out at The Biological Activity Laboratory, the Central Laboratory, Universitas Padjadjaran, Bandung, Indonesia: the cells were cultured on RPMI media (Sigma-Aldrich) containing 10% FBS and antibiotics, and trypsin-EDTA was added and incubated for 5 minutes. When the growth of cells reached confluent level where the numbers of cell lines were minimum 70%, they were then transferred to 96-microtubewell plates, and each of the microtubewas added with samples of various concentrations and incubated for 48 hours. The mixture was then added with preto blue gluing as cell staining and incubated for 1-2 hours until a discoloration was observed. PrestoBlue® reagent is reduced by the blue compound resazurin to resorufin, which is pink and very fluorescent. The absorbance measurements were carried out at 570 nm (resorufin) and 600 nm (resazurin) wavelengths using a multimode reader, and cisplatin was used as a positive control and DMSO as a negative control.17

The anticancer activity against HCC 1995 and MDA-MB 231 cell lines was analyzed using the MTT assay method which were carried out at The Culture Cell and Cytogenetics Laboratory, Medical Faculty, Universitas Padjadjaran, Bandung, Indonesia: the cells were cultured in RPMI 1640 media (Sigma-Aldrich) containing 10% fetal calf serum, antibiotics, and streptomycin. The cells and media were incubated for 24 hours, and the cells were then added with samples with various concentrations and phosphate buffer saline. Furthermore, the mixture was then incubated again for 24 hours, and 3-(4,5-Dimetiltiazol-2-il)-2,5-diphenyltetrazolium bromide (MTT) compound was added to each well containing 15,000 cells and incubated for 2 hours. The MITT reaction was measured using n-hexane, and the absorbance of the reaction was measured using an ELISA reader at a wavelength of 550 nm.18

The anticancer activity of MCF-7 breast adenocarcinoma (ATCC HTB-22) and A549 Lung Carcinoma (ATCC CCL-185) cell lines was analyzed using the MTS assay method which were carried out at The Biological Activity Laboratory, the Central Laboratory, Universitas Padjadjaran, Bandung, Indonesia: the cells were cultured on RPMI media (Sigma-Aldrich) containing 10% FBS and antibiotics, and trypsin-EDTA was added and incubated for 5 minutes. When the growth of cells reached confluent level where the numbers of cell lines were minimum 70%, they were then transferred to 96-microtubewell plates, and each of the microtubewas added with samples of various concentrations and incubated for 48 hours. The mixture was then added with preto blue gluing as cell staining and incubated for 1-2 hours until a discoloration was observed. PrestoBlue® reagent is reduced by the blue compound resazurin to resorufin, which is pink and very fluorescent. The absorbance measurements were carried out at 570 nm (resorufin) and 600 nm (resazurin) wavelengths using a multimode reader, and cisplatin was used as a positive control and DMSO as a negative control.17

The anticancer activity against HCC 1995 and MDA-MB 231 cell lines was analyzed using the MTT assay method which were carried out at The Culture Cell and Cytogenetics Laboratory, Medical Faculty, Universitas Padjadjaran, Bandung, Indonesia: the cells were cultured in RPMI 1640 media (Sigma-Aldrich) containing 10% fetal calf serum, antibiotics, and streptomycin. The cells and media were incubated for 24 hours, and the cells were then added with samples with various concentrations and phosphate buffer saline. Furthermore, the mixture was then incubated again for 24 hours, and 3-(4,5-Dimetiltiazol-2-il)-2,5-diphenyltetrazolium bromide (MTT) compound was added to each well containing 15,000 cells and incubated for 2 hours. The MITT reaction was measured using n-hexane, and the absorbance of the reaction was measured using an ELISA reader at a wavelength of 550 nm.18
(C-4'); 146.6 (C-3'); 164.6 (C-9); 165.8 (C-7); and 193.2 (C-4). UPLC-QTOFMS m/z 287.28 [M+] (calcd. for C_{15}H_{12}O_{6}, m/z 288.25).

Garbanzole (3) (Figure 1): yellow needle crystal. ¹H-NMR (in acetone-d₆, 500 MHz) ¹H (ppm): 7.70 (1H, d, J = 8, H-5); 6.60 (1H, dd, H-6); 6.37 (1H, d, H-8); 7.41 (2H, d, H-2' and 6'); 6.87 (2H, d, H-3' and H-5') 5.02 (1H, d, H-2); and 4.45 (1H, d, H-3). ¹³C-NMR (in acetone-d₆, 125 MHz): ¹³C (ppm): 84.9 (C-2); 73.9 (C-3); 193.3 (C-4); 129.8 (C-5); 111.8 (C-6); 165.9 (C-7); 103.7 (C-8); 164.6 (C-9); 113.1 (C-10); 129.4 (C-1'); 130.4 (C-2' and C-6'); 115.9 (C-3' and C-5'); and 158.9 (C-4'). UPLC-QTOFMS m/z 273.0559 [M+2] (calcd. for C_{15}H_{12}O_{6}, m/z 272.0559).

Methyl gallate (4) (Figure 1): yellow needle crystal. ¹H-NMR (in acetone-d₆, 500 MHz) ¹H (ppm): 3.75 ppm (3H, s, -OCH₃); 7.07 (2H, s, H-2 and H-6); and 8.19 (1H, s, -OH). ¹³C-NMR (in acetone-d₆, 125 MHz): ¹³C (ppm): 166.3 (C-7); 145.2 (C-3 and C-5); 137.9 (C-4); 120.9 (C-1); 108.9 (C-2 and C-6); and 51.1 (-OCH₃). UPLC-QTOFMS m/z 183.09 [M-] (calcd. for C_{15}H_{11}O_{5}, m/z 184.15).

Compound 1 is a colorless crystal. The UPLC-QTOFMS spectrum for compound 1 has the molecular formula C_{29}H_{48}O, m/z 412.69. The


1H-NMR spectrum of isolate 1 is similar to the reference compound reported by others and it showed a typical signal for the group of steroid compound in which the signal accumulated in the area below 2 ppm was typical for steroids. Four signals in the 1H region (3H, J = 9.74 Hz; 0.81 (3H, s); 0.82 (3H, m); and 0.79 (1H, s) ppm indicated the presence of a methyl signal bound to C-21, C-26, C-27 and C-29, respectively. Furthermore, it is believed that 9 signals indicated the presence of methylene protons in the 1H region of 1.82 (2H, m); 1.51 (2H, m); 2.26 (2H, J = 6.5 Hz); 1.97 (2H, m); 1.50 (2H, m); 1.97 (2H, m); 1.52 (2H, m); 1.23 (2H, m); 1.15 (2H, m) ppm, which is binds to C-1, C-2, C-4, C-7, C-11, C-12, C-15, C-16 and C-28 respectively. The proton signal from methine is believed to appear at 1.47 (1H, m); 0.95 (1H, m); 1.05 (1H, m); 1.15 (1H, m); 1.97 (1H, m); 1.47 (1H, m); and 1.81 (1H, m) ppm bound to C-8, C-9, C-14, C-17, C-20, C-24, and C-25, respectively. There is a typical signal for olefinic protons at 5 ppm and there is a signal for oxygenated protons at 3 ppm, which is commonly reported in the class of the steroid compound. The signal was detected as an oxygenated proton at 3.51 (1H, m) ppm. The signal in the region 5.13 (1H, d); 5.12 (1H, m) and 5.15 (1H, m) ppm are from the olefinic methine double bond protons attached to C-6, C-20 and C-21, respectively.

Rings A, B, and C consist of six carbon or cyclohexane atoms, and ring D consists of five or cycloheptane. Furthermore, most of the steroids have properties, which includes the oxygen functional group (as = O or OH) at C-3, and contains side groups at C-17, many of which contain double bonds at C-4 - C-5 or C-5 - C-26. The carbon signal that appears in the area above 100 ppm, is 140.9; 120.7; 138.5; and 129.4 ppm, which are at C-5, C-6, C-22, and C-26. The double bond was confirmed by the presence of two double bonds in the analyzed compound. The double bond signal is reported in the 140.9 region; 120.7; 138.5; and 129.4 ppm. The methyl signal is believed to be in the 12.2 (C-18) region; 19.5 (C-19); 23.2 (C-21); 20.0 (C-26); 21.2 (C-27) and 12.2 ppm. The methyl signal is believed to present in the region: 36.3 (C-1); 32.5 (C-2); 42.3 (C-4); 32.0 (C-7); 21.4 (C-11); 39.2 (C-12); 24.5 (C-15); 29.2 (C-16) and 25.6 (C-28). In addition, the signal in the region 32.0 (C-8); 50.3 (C-9); 56.9 (C-14); 56.2 (C-17); 39.9 (C-20); 51.4 (C-24), and 31.8 ppm were believed to indicate the presence of a methine group.

The quaternary carbon group containing 3 signals was predicted in the 140.9 (C-5) region; 36.5 (C-10) and 42.4 (C-13) ppm. Figure 1 shows that the methyl proton at position C-29 0.78 correlates with C-26 (0.20) and C-28 (25.6). The methyl proton at position C-18 (0.66) correlates with C-12, C-13, C-14 and C-17 with each value 39.2, 42.4, 56.9; and 56.2 ppm. The methyl proton at position C-19 (0.99) correlates with C-1, C-5, C-9, and C-10 with values of 36.3, 141, 50.1 and 50.1.
Fig. 5. The comparison of anticancer activity test for all compounds isolated.
showed 8 signals of methine carbon (δc 74.0; 85.0; 103.1; 111.8; 115.8; 115.9; 120.9; 129.8 ppm) and there were 7 carbon signals quaternary (δc 113.1; 130; 1; 145.8; 146.6; 164.6; 165.8; 193.2 ppm). The 13C-NMR spectrum shows a signal at a shift below 100 ppm, namely at δc 85.0 and 74.0 ppm as a characteristic of saturated carbon sp3 which binds to electronegative atoms such as oxygen. The carbon signal at 193.2 ppm shift is characterized by carbonyl carbon, which has a shift range between 185-220 ppm. The 2D NMR COSY spectrum of 1H-1H correlation shows that there is a correlation between H-3 (δH 4.53 ppm) with H-2 (δH 5.00 ppm) and H-5 (δH 7.72 ppm) with H-6 (δH 6.62 ppm). This confirms that the basic structure of the isolate is a flavonoid with 2 protons in ring A and ring B, each of which is correlated. The results of the 2D NMR HMBC spectrum analysis showed that there were 8 correlations between the protons and the carbon signal. The correlation shows a direct bond between protons and carbon, namely the proton carbon signal. The correlation shows a direct bond were 8 correlations between the proton and the carbon signal. The 13C-NMR HMQC spectrum analysis showed that there were 7 methine carbon signals quaternary (δc 113.1; 129.8 ppm and δc 130.4 ppm) and 6 quaternary carbon signals (δc 113.1; 129.4; 158.9; 164.6; 165.9 and δc 193.3 ppm). In the spectrum, a signal appears at 193.3 ppm, which indicates the type of carbonyl carbon (C = O). The 13C-NMR spectrum shows that there are 10 carbon signals, which ranges from 100-167 ppm that are believed to be aromatic carbon. Furthermore, HMQC 2D spectrum shows a direct correlation between protons and carbon. The correlation signal appears up to 7 signals at δc 130.4 ppm (C-2'); 129.8 (C-5') ppm, respectively correlated with carbon at δc 73.9 (C-3), respectively; 193.3 (C-2); 103.7 (C-8); 111.8 (C-6); 115.9 (C-3'/5'); and 129.8 (C-5') ppm. 2D HMBC analysis was performed, and the structure of the isolate was believed to be a flavonoid. This is reinforced by the signal that appears at δc 5.04 ppm (C-2), which correlates with δc 73.9 (C-3); 193.3 (C-4); 129.4 (C-1'); 130.4 (C-2'/6'). This signal shows the correlation between the C and B rings of flavonoids. The important HMBC correlation of 3 was shown in Figure 4. Based on the 1H-NMR spectrum of compound 4, there is CH3 (-OCH3), which is oxygenated at δH 3.75 ppm (3H, s). A typical aromatic signal appears at δH 7.07 (2H, s) with a symmetrical plane, hydroxy proton (-OH) appears at δH 8.19 (1H, s). Seven carbon signals that are a C = O signal at δc 167.9 ppm (indicating the presence of carbon ester), one signal indicates the presence of aromatic carbon (C=OH) at δc 145.2 ppm, at δc 137.9 ppm indicates aromatic carbon (C-OH), δc 120.9 contains aromatic carbon (C-C), δc 108.9 ppm contains aromatic carbon (C-H), at δc 51.1 indicates the presence of –OCH3. These values are in agreement with reported values available in the literature.

Anticancer Activity of Extracts and Compounds 1-4

The anticancer activity against MCF-7 and A549 was measured using the MTS assay method, while the anticancer activity of HCC-1954 and MDA-MB 231 cells was measured using the MTT assay method and the results of the anticancer activity test are shown on the Table 1 and the comparison of their IC50 values are shown in Figure 5.

All compounds 1 – 4 were assayed for their anticancer property against MCF-7, A549, HCC-1954 and MDA-MB231 cell lines. All isolated compounds exhibited moderate anticancer activity against almost cell lines tested. Compounds 2 and 4 were more active on HCC-1954 cell with IC50 values of 134.35 ± 44.62 and 153.69 ± 12.54 µg/
A549, and HCC-1954 cell lines with IC50 values respectable anticancer activity against MCF-7, important for anticancer activity.27 carbonyl group at C-4 on the flavan skeleton is very may be due to its chiral structure. In addition, the compounds, compound 3 gave the most active in the anticancer activity test against MDA-MB-231 cell line with IC50 value of 233.41 ± 91.57 µg/mL. While compounds 2 and 3 demonstrated respectable anticancer activity against MCF-7, A549, and HCC-1954 cell lines with IC50 values ranging from 134.35 ± 44.62 to 568.77 ± 98.13 µg/mL. The compounds 2 and 3 are flavanones containing a chiral carbon on chroman-4-one ring unit which is flexible. According Woo et al., 28 the wide range of the flavanone bioactivity may be due to its chiral structure. In addition, the carbonyl group at C-4 on the flavan skeleton is very important for anticancer activity.27

However, the structure-activity relationship study is required to provide better understanding of their anticancer activity. The results of the anticancer test for the compounds isolated in this work are lower compared to other compounds reported by others both in the synthetic compounds such as organotin (IV) carboxylates29, 30 or other isolated compounds from other plants31, although the cell lines used were different. However, the results reported in this work are believed still very important results in attempts to find new candidate for anticancer drugs.

CONCLUSIONS

Four compounds namely stigmasterol (1), fustin (2), garbanzol (3), and methyl gallate (4) were successfully isolated from the stem of B. macrophylla. These compounds were well characterized and the characterization data obtained were similar to the known compounds previously published. These compounds were tested for their anticancer activities against 4 cell lines. The result showed based on the IC50 values of compounds 2 and 4 were more active on HCC-1954 cell with IC50 values of 134.35 ± 44.62 and 153.69 ± 12.54 µg/mL, respectively. The compound 3 was the most active against MDA-MB-231 cell line with IC50 value of 233.41 ± 91.57 µg/mL.

Data availability

Data can be made available upon request from the corresponding author.

REFERENCES

1. Lim TK. Bouea macrophylla. In: Edible Medicinal and Non-Medicinal Plants. Springer. Dordrecht.: 69-71 (2012).
2. Hanifa D, Susilawati Y. Review artikel: Potensi tanaman gandaria (Bouea macrophylla Griff) sebagai obat herbal yang beraktivitas antioksidan. Farmaka.; 15(3): 134-142 (2017). https://doi.org/10.24198/jf.v15i3.13559 (In Indonesian)
3. Rajan NS, Bhat R. Antioxidant compounds and antioxidant activities in unripe and ripe kundang fruits (Bouea macrophylla Griffith). Fruits.; 71(1): 41–47 (2016). https://doi.org/10.1051/fruits/2015046
4. Thummajitsakul S, Silprasit K. Genetic differentiation and antioxidant activities of Bouea macrophylla Griffith in Nakhon Nayok province. J. Appl. Biol. Chem.; 60(1): 41-47 (2017). https://doi.org/10.3839/jabc.2017.008
5. Rajan NS, Bhat R. Volatile constituents of unripe and ripe kundang fruits (Bouea macrophylla Griffith). Int. J. Food. Prop.; 20(8): 1751-1760 (2017). https://doi.org/10.1080/10942912.2016.1218892
6. Putri NEK. Aktivitas Antioksidan Ekstrak Metanol Kulit Buah Ramania (Bouea macrophylla Griff) dengan Metode DPPH. Proc. Mulawarman Pharm. Conf.; 7: 28-31 (2018). https://doi.org/10.25026/mpc.v7i1.287 (In Indonesian).
7. Sukalingam K. Preliminary phytochemical analysis and in vitro antioxidant properties of Malaysian “Kundang” (Bouea macrophylla Griffith). Trends Phytochem. Res.; 7(1): 261-266 (2018).
8. Kulsum S, Suresh A, Mehta A. Correlation of Antioxidant and Antiproliferative Activity of Amla and Ginger. Asian J Pharm Clin Res.; 11(8): 263–269 (2018). https://doi.org/10.22159/ajpcr.2018.v11i8.26073
9. Andina L, Musfirah Y. Total phenolic content of Malaysian ‘Kundang’ (Bouea macrophylla Griffith) seeds: proximate composition, HPLC fingerprint, and antioxidant, anticancer and antimicrobial properties of ethanolic seed extracts. Heliyon;
18. Ciapetti G, Cenni E, Pratelli L, Pizzoferrato P, Dechesna N. Pentagalloyl Glucose- and Ethyl Gallate-Rich Extract from Maprang Seeds Induce Apoptosis in MCF-7 Breast Cancer Cells through Mitochondria-Mediated Pathway. *Evid.-Based Compl. Alt. Med.*; 2020: 1-19 (2020). https://doi.org/10.1155/2020/5686029.

19. Rudiana T, Suryani N, Indritanoto DD, Usamsyah Y, Amelia A, Noviany N, Hadi S. Characterization of antioxidative fraction of plant stem *Bouea macrophylla* Grif. *IOP J. Phys.: Conf. Ser.;* 1341(17): 1-8 (2019). https://doi.org/10.1088/1742-6596/1341/7/072008.

20. Noviany N, Nurhidayat A, Hadi S, Suhartati. Determination and Interpretation of NMR Spectra of Flavonoids. In: The Systematic Identification of Flavonoids. Springer: Berlin, Heidelberg, Germany, 253-273 (1970). https://doi.org/10.1007/978-3-642-88458-0_8.

21. Mabry TJ, Markham KR, Thomas MB. The Determination and Interpretation of NMR Spectra of Flavonoids. In: The Systematic Identification of Flavonoids. Springer: Berlin, Heidelberg, Germany, 253-273 (1970). https://doi.org/10.1007/978-3-642-88458-0_8.

22. Pavia DL, Lampman GM, Kriz GS, Vvyyan JR. Introduction to Spectroscopy (4th ed.). Brooks/Cole: Belmont, CA, USA, 2009.

23. Hashida K, Tabata M, Kuroda K, Otsuka Y, Kubo S, Mekino Y, Tono Y, Tonosaki M, Ohara S. Phenolic Extractives In The Trunk of Toxicodendron Vernicifluum: Chemical Characteristics, Contents and Radial Distribution. *J Wood Sci.* 60: 160-168 (2014). https://doi.org/10.1007/s10086-013-1385-8.

24. Lee TH, Chiu JH, Lee WK. Separation and Determination of Chemical Constituents in the Roots of *Rhus javanica* L var. *roxburghiana*. *J. Chinese Chem. Soc.;* 52: 833-841 (2005). https://doi.org/10.1007/jcsc.200500117.

25. Chiang E, Tan Y, Lim C. Potent Quorum Sensing Inhibition by Methyl Gallate Isolated From Leaves of *Anacardium occidentale* L. (Cashaw). *Chiang Mai J. Sci.;* 42(3): 650-656 (2015).

26. Fotso GW, Kamgba J, Ngameni B, Uesugi S, Ohno M, Kuroda K, Momma H, Kubo S, Komuro Y, Furuno H, Shiono Y, Igrid SK, Yeboah SO, Ngadjui BT. Secondary metabolites with antiproliferative effects from *Albizia glabrata* and *Albizia glutinosa* var. *glomerata* Oliv. (Mimosoideae). *Nat. Prod. Res.* 31(17): 1981-1987 (2017). https://doi.org/10.1080/17437090.2016.1210899.

27. Ciapetti G, Cenni E, Pratelli L, Pizzoferrato A. In vitro evaluation of cell/biomaterial interaction by MTT assay. *Biomaterials.;* 14(5): 359-364 (1993). https://doi.org/10.1016/0142-9693(93)90055-7.

28. Forgo P, Kover KE. Gradient enhanced selective experiments in the 1H NMR chemical shift assignment of the skeleton and side-chain resonances of stigmasterol, a phytosterol derivative. *Steroids.;* 69(1): 43-50 (2004). https://doi.org/10.1016/j.steroids.2003.09.012.

29. Darwati D, Nurilestari N, Mayanti T, Isolasi Senyawa Steroid dari Akar Tumbuhan Asam Kandid (Garcinia cowa Roxb. ex DC ) Sebagai Obat Penurun Demam. *J. Penel. Hasil Hutan.;* 37(1): 51-57 (2019). https://doi.org/10.20886/j.phh.2019.37.1.51-57 (in Indonesian)

30. Shrestha S, Lee D, Park J, Cho JG, Lee DS, Li B, Kim YC, Jeon YJ, Yeon SW, Baek NI. Flavonoids from the Fruits of Nepalese *Suman (Rhus parviflora)* Attenuate Glutamate-induced Neurotoxicity in HT22 Cells. *Food Sci. Biotechnol.* 22: 895–902 (2013). https://doi.org/10.1007/s10068-013-0161-2.
31. Suhartati T, Hernawan, Suwandi JF, Yandri Y, Hadi S. Isolation of Artotin E from the Root Bark of Artocarpus rigida, Synthesis of Artotin E Acetate and Evaluation of Anticancer Activity. *Maced. J. Chem. Chem. Eng.* 37(1): pp. 35-42 (2018). https://doi.org/10.20450/mjcece.2017.1406

32. Suhartati T, Epriyanti E, Borisha I, Yandri Y, Suwandi JF, Qudus HI, Yuwono SD, Hadi S. In Vivo Antimalarial Test of Artocarpin and in vitro Antimalarial Test of Artotin M Isolated from Artocarpus. *Rev. Chim.* 71(5): 400-408 (2020). https://doi.org/10.37358/RC.20.5.8150