Research Article

# *In Vitro* Study of the Alkaloid Anticancer Compound From Makassar Medicinal Plants *Boehmeria virgata* Linn

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**ABSTRACT**

Plant *Boehmeria virgata* belongs to the family Urticaceae and has been traditionally used by Makassar tribe to treat cancer. In the previous study, we showed that BVI03 isolate has potent antiproliferative activity against HeLa cells. The aim of the research was to isolate and characterize the bioactive compounds of BVI03 and to measure its anti-proliferative activity against HeLa cells. The structure of the compound was characterized by using IR, 1H and 13C NMR and MS spectroscopy method. The compounds was also evaluated its antiproliferative activity against HeLa cell line by MTT assay. The BVI03 compound was characterized as alkaloid compound namely 10- (6,6-dihydroxy-hexyl) -2,3,6-trimethoxy-phenanthrene-9-carboxylic acid amide. The antiproliferative activity of the compound was measured against cervix cancer HeLa cell line with the IC50 value of 39.05 at ug/ml, in compare with IC50 of Doxorubicin as positive control namely 56.56 ug/ml. From the study we showed that the alkaloid compound BVI03 from *Boehmeria virgata Linn* has anticancer activity against HeLa cell line is a candidate to be evaluated clinically, moreover, it had better anticancer activity than positive control doxorubicin.

**Keywords:** *Boehmeria virgata*, HeLa cell line, BVI03, Makassar tribe, antiproliferative activity.

## INTRODUCTION

Cancer is the second leading cause of death after cardiovascular disease. Globally, the number of cancer deaths is projected to increase from 7.1 million in 2002 to 11.5 million in 2030[1]. Based on interviews of basic health research in 2013 the prevalence of cancer in the population of all ages in Indonesia is 1.4% and cervical cancer is the highest cancer prevalence of 0.8%[2] Recently, many advances have been made in the development of surgical procedures, radiotherapy and chemotherapeutic agents, including the case of combining chemotherapy and hormone therapy with immunotherapy[3]. One of the main problems that need to be overcome in cancer treatment is that a tumor should be considered as a heterogeneous multiple cell subpopulation. Another aspect that should be taken into account is the development of resistant phenotypes, which include cytotoxic resistance to anticancer compounds and/or resistance to pro-apoptotic stimuli. All these factors must be considered to develop effective therapies for cancer. Despite all efforts to prevent oncological disorders and to develop new therapies, the cancer rate persists worldwide. Thus, there is an increasing emphasis on strategies to optimize tumor control, prolong survival, minimize chemotherapy side effects and improve quality of life for patients[4] . From a research point of view, this situation also demands the development of new drug discovery strategies and molecules.

Plant *Boehmeria virgata* belongs to the family Urticaceae and has been traditionally used by Makassares tribe to treat cancer[5]. Parang Romang medicinal plant along with three other plants, namely *Ilicifolius Acanthus* Linn, *Acalypha indica* and Eupatorium odoratum L. have been investigated their activities against HeLa cells and was found that *B. virgata* has the most potent activity with $IC_{50}$ values are 9.40 □g/ml[6]. Wardihan *et al.* (2013) mention that the ethanol extract of *Boehmeria virgata* is active against HeLa cells, Widr, T47D and Vero cell lines with $IC_{50}$ values are 18.99 ± 0.234; 18.925 ± 1.277; 12.732 ± 0.945, and 16.022 ± 0.663 ug/ml, respectively[7]. Hexane, ethyl acetate and methanol extract also have antiproliferation activity against human bladder 5637 cancer cells with $IC_{50}$ values respectively 1.4; 3.96 and 2.18 ug/ml[8]. In addition Manggau *et al*. (2012b) also reported BVI03 isolate has potent antiproliferative activity ($IC_{50}$ = 0.126 ug/ml) against HeLa cells[9].

There is not much information about the bioactive compounds from this plant, but some studies have shown that the genus Boehmeria has active compounds against several cancer cells. Some of them are cryptopleurine isolated from *B. cylindrica* exhibit very high activity in human epidermoid carcinoma of the nasopharynx (KB) test system (ED5010-5 ppm)[10] and AGS human gastric cancer cells with $IC_{50}$ = 8.7 nM[11]. Boehmeriasins A, a phenanthroquinolizidine alkaloid isolated from *B. siamensis* showed cytototoxic activity against 12 types of cancer cells from 6 panel $GI_{50}$ between 0.2 to 100 ng/mL[12], 3 cancer cells (CEM, HeLa and L1210) and 2 endothelial cells (HMEC-1, BAEC)[13] and was active against breast cancer cells MDA-MB-231[14].

Based on the previous data, *Boehmeria virgata* plant has a big potential for further investigation in the search for new anticancer agents. With the reports of several active fractions of the plant as well as the discovery of active

isolates that have been characterized as alkaloids, namely BVI03 (IC50 = 0.126 ppm)[9]. Alkaloid itself is known as an anti-cancer agent class of plants that are generally most frequently used clinically in the treatment of cancer, and some researches suggest that alkaloids compounds of the genus Boehmeria active against various types of cancer cells. So the researchers wanted to characterize compounds of parang romang (*B. virgata*) leaves that have anti-proliferation activity against HeLa cells.

## MATERIALS AND METHODS

### General procedure

All samples were fractionated and isolated using vacuum liquid chromatography (VLC), thin layer chromatography (TLC) and radial chromatography using chromatotron models 7924T (T-Squared Technology Inc.). Silica 60 p.a GF254 (E. Merck) was used as the stationary phase for VLC and silica 60 PF254 containing gypsum (E. Merck) as a stationary phase for chromatotron. Spectrophotometer FTIR (Shimadzu), nuclear magnetic resonance (NMR) spectra Spectrometer were obtained from a JEOL spectrometer.

### Plant material

The leaves of parang romang (*B. virgata*) were collected from Tamalatea village Manuju District of Gowa in South Sulawesi. The collected leaves were air dried at room temperature until their use.

### Isolation of compound from parang romang (*B. virgata*) leaves

Mashed parang romang leaves (10 kg) were macerated with methanol (MeOH) 60 L for 4 x 24 hours. The extract was concentrated by rotary vacuum evaporator (188.48 g). Thirty grams of methanol extract was fractionated by VLC using a column with diameter of 13 cm, as adsorbent Si-gel (250 g) was used and a mixture of eluent hexane-ethyl acetate (8: 2; 7: 3; 5: 5; 3: 7; ethyl acetate 100%, methanol 100 %) were used to obtain 4 fractions: A (24.08 g); B (35.57 g); C (19.31 g) and D (58.50 g). Furthermore, D fractions were then separated until we got brownish-yellow crystals (8.5 mg) that is known as BVI03 isolates.

### Structure determination

The structure of the compound was characterized by using $^{1}H$ and $^{13}C$ NMR spectroscopy method. Spectra of $^{1}H$ NMR ($CDCl_3$, 500 Hz) $\delta_H$ 7.33 (1H, *d*, 9 Hz, H-8); 7.89 (1H, *s*, H-1); 9.24 (1H, *d*, 9 Hz, H-7); 7.91 (1H, *s*, H-4); 7.89 (1H, *s*, H-5); 4.86 (1H, *t*, H-15); 4.06 (3H, *s*, MeO-6); 4.13 (3H, *s*, MeO-3); 4.04 (3H, *s*, MeO-2); 3.66 (1H, *m*, H-10); 3.00 (1H, *t*, H-10); 2.05 (1H, *m*, H-14); 1.56 (1H, *m*, H-14); 2.36 (2H, *m*, H-11); 1.18 (2H, *m*, H-12); 1.33 (2H, *m*, H-13) dan 7.41 (2H, *s,* $H_2N$-9).

Spectra of $^{13}C$ NMR 170.0 (C-9); 159.4 (C-6); 150.8 (C-2); 150.2 (C-3); 136.2 (C-10a); 132.9 (C-8b); 127.6 (C-8); 127.0 (C-4b); 124.7 (C-4a); 121.3 (C-10b); 120.9 (C-8a); 116.4 (C-1); 104.8 (C-7); 104.7 (C-4); 104.2 (C-5); 91.8 (C-15); 56.2 (MeO-6); 56.2 (MeO-3); 55.7 (MeO-2); 39.9 (C-10); 37.3 (C-14); 29.9 (C-11); 26.0 (C-12) dan 14.3 (C-13).

### Cell cultures

HeLa cell line were cultured in RPMI 1650, WiDr was cultured in Dulbecco's Modified Eagle Medium (DMEM®). All cells were subcultured after mild trypsinization with typsin-EDTA (Sigma-Aldrich, USA), trypan blue dye (Sigma-Aldrich®, USA) exclusion assay was performed to determine the cell number and viability.The media (Sigma-Aldrich®, USA) was supplemented at 10% with fetal bovine serum (Gibco®) and streptomycin plus penicillin (100 ug/ml and 100 ug/ml, respectively; Sigma-Aldrich®, USA). The cell line were kept at 37ºC, 98% relative humidity with 5% CO2 atmosphere.

### Cytotoxic assay- MTT Method

HeLa cells (5 x $10^3$ cells/well) were transferred into 96-well plate and incubated for 24 hours (confluent 80%). The cells were treated by isolate BVI03 and doxorubicin (Ebewe). At the end of the treatment incubation, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) 10 µl were added to each well followed by 4 hours incubation in 37°C chamber. Viable cells react with MTT to form purple formazan crystals. After 4 hours, stopper SDS was added to dissolve the formazan crystal. Following overnight incubation (with protection from light exposure), the cells were shaken for 10 minutes before being read by Elisa reader at λ 595 nm. The obtained absorbance of each well converted to the percentage of viable cell:

% of cell death =

$$\frac{(A\ cell\ control - A\ \ media) - (A\ sample - A\ media)}{(A\ cell\ control - A\ \ media)} \times 100\%$$

## RESULTS AND DISCUSSION

### Structure determination

In this study, a compound was isolated from the leaves of *Boehmeria virgata* (parang romang) plants, hereinafter referred as BVI03[9].

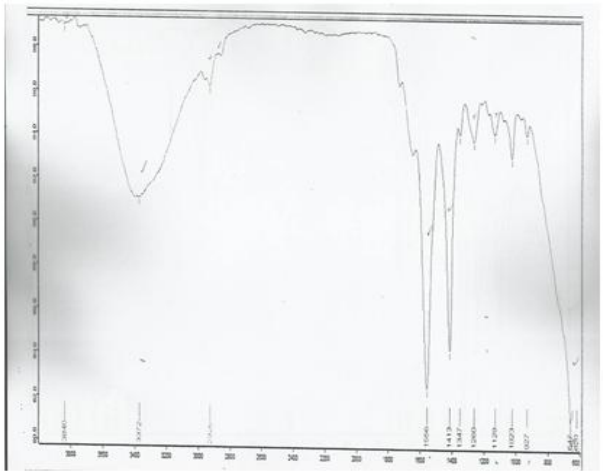

**Figure 1:** IR spectra data of the BVI03 Isolate

At the IR spectrum data, as shown in figure 1, O-H stretching groups are shown at 3372 $cm^{-1}$. The amine groups showed medium absorption bands at 927 $cm^{-1}$ and primer amine groups showed strong absorption band at 1556 $cm^{-1}$. CH-aliphatic was detected at 2926 $cm^{-1}$. Aromatic groups showed strong absorption bands at 1413 $cm^{-1}$, whereas alkene groups showed strong absorption bands at 647 $cm^{-1}$,620 $cm^{-1}$ and weak at 1129 $cm^{-1}$.

In the table 1 we show the $^{1}H$ and $^{13}C$ NMR spectroscopy data of the isolate BVI03.

BVI03 compound was isolated as brownish-yellow crystals. Table 1 shows that there are 24 carbon in the compound with a chemical shift between 100-160 which is Csp2 carbon. The carbons can be paired which form conjugated double bonds as phenanthrene. This is also supported by 1H-NMR spectral data that showed the higher chemical shift 9.24; 7.91; 7.88; 7.41 and 7.33 ppm. Carbon with chemical shift 150.2; 150.8 and 159.4 ppm, is thought to be carbon Csp2 that can form a bond with oxygen as Csp2-OH or Csp2-OR. Electronegativity influence also occurs in carbon with chemical shift 56.2; 56.1 and 55.7 ppm predicted a methyl group that has a high chemical shift caused by the atom more electronegative (oxygen). A methyl group and the oxygen will form the methoxy (-O-CH3) and possibly a pair of three carbon Csp2 to form Csp2-O-CH3.

**Table 1:** Spectrocopy $^{1}H$ dan $^{13}C$ NMR data of isolate BVI03

| Position | $\delta_C$ | $\delta_H$ (*mult, J*, ΣH) | Functional group estimated | Reference |
|---|---|---|---|---|
| 9 | 170,0 | - | Carbon at $RCONH_2$, chemical shift (δ) between 150-178 ppm. Carbon with δ between 100,0-160,0 ppm were olefinic carbon or $Csp^2$ carbon (a) Protons with coupling constant (J) 6-10 Hz were orto position(b) -C-OH δ = 45-94 ppm. C-O-C between 50-80 ppm (a) δ proton metoxi 3,2-4,3 ppm (b). δ proton bending amide 5-8,8 ppm. δ 15-55 ppm were $Csp^3$ in alifatic carbon (a). | (a) Silverstein, 2002 (b) Fessenden and Fessenden, 1997 (c) Susi, 2010 |
| 6 | 159,4 | - | | |
| 2 | 150,8 | - | | |
| 3 | 150,2 | - | | |
| 10a | 136,2 | - | | |
| 8b | 132,9 | - | | |
| 8 | 127,6 | 7,33 (*d*, 9 Hz, 1H) | | |
| 4b | 127,0 | - | | |
| 4a | 124,7 | - | | |
| 10b | 121,3 | - | | |
| 8a | 120,9 | - | | |
| 1 | 116,4 | 7,89 (*s*, 1H) | | |
| 7 | 104,8 | 9,24 (*d*, 9 Hz, 1H) | | |
| 4 | 104,7 | 7,91 (*s*, 1H) | | |
| 5 | 104,2 | 7,89 (*s*, 1H) | | |
| 15 | 91,8 | 4,86 (*t*, 1H) | | |
| MeO-6 | 56,2 | 4,06 (*s*, 3H) | | |
| MeO-3 | 56,2 | 4,13 (*s*, 3H) | | |
| MeO-2 | 55,7 | 4,04 (*s*, 3H) | | |
| 10 | 39,9 | 3,66 (*m*, 1H) | | |
| | | 3,00 (*t*, 1H) | | |
| 14 | 37,3 | 2,05 (*m*, 1H) | | |
| | | 1,56 (*m*, 1H) | | |
| 11 | 29,9 | 2,36 (*m*, 2H) | | |
| 12 | 26,0 | 1,18 (*m*, 2H) | | |
| 13 | 14,3 | 1,33 (*m*, 2H) | | |
| $H_2N$-9 | - | 7,41 (*s*, 2H) | | |

This is supported also by the presence of protons with chemical shift 4.06; 4.04 and 4.13 ppm which is likely to further support the methoxy protons in their approximate 3 methoxy groups on the compound phenanthere. Proton on carbon RCONH2 have chemical shift between 150-178 ppm[14], the results of measurements are 13C-NMR chemical shift of carbon at 170.0 ppm indicating a carbonyl group bound to nitrogen (amide), it is supported also by the presence of protons in chemical shift 7.41 ppm are probably the amide proton. Carbon bound alcohol (-C-OH) had chemical shift 45-94 ppm[14], the chemical shift of 91.8 ppm possibility of a second bound carbon atom with a large electronegativity (such as oxygen). Chemical shift between 15-55 ppm were Csp3 carbon on a alifatic/cyclic, this suggests that the carbon with chemical shift 39.9; 37.3; 29.9; 25.9; and 14.9 ppm were Csp3 carbon in linear chain. 1H-NMR spectral data also indicate a buildup of multiplicity proton which is multiplet (m) and triplet (t) of the proton with chemical shift 3.66; 3.00; 2.08; 2.05; 2.04; 1.52 and 1.59 ppm

indicating that many proton neighbors, besides chemical shift protons were also quite close together. This indicates that the structure isolates bound have a straight chain alkane. Thus allegedly BVI03 compound is 10-(6,6-dihydroxy-hexyl)-2,3,6-trimethoxy-phenanthrene-9-carboxylic acid amide as shown in figure 2.

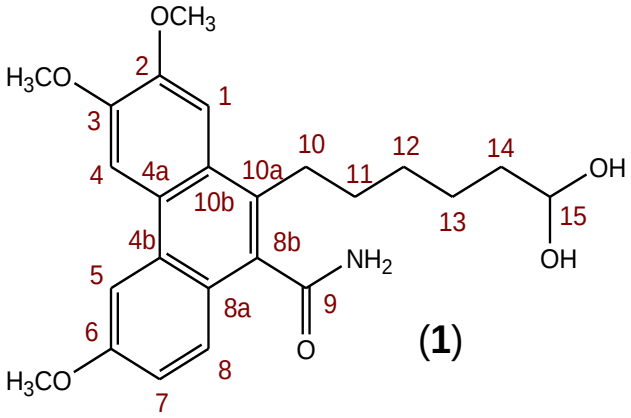


**Figure 2**: *tentative structures isolate* BVI03

The compound obtained is a novel compound that allegedly required a variety of additional data such as two-dimensional NMR, mass spectroscopy and IR to ensure that the proposed structure of the compounds.

**Anticancer Activity against HeLa cell Line**

The result data of the putative compound 10-(6,6-dihydroxyhexyl)-2,3,6-trimethoxyphenanthrene-9-carboxamide as anticancer against HeLa cell line using MTT method can be shown in table 2 and figure 3.

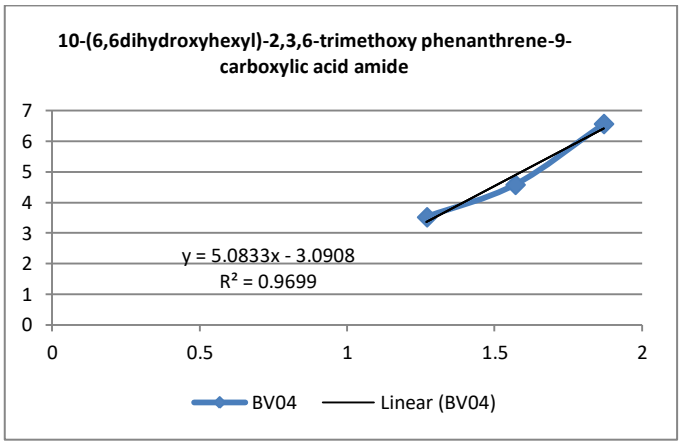


**Figure 3:** Log graph of the concentration of 10-(6,6dihydroxyhexyl) -2,3,6-trimethoxyphenanthrene-9-carboxylic acid amide with a value of probit

**Table 2:** Anticancer activity of 10-(6,6 hydroxy hexyl)-2,3,6-trimethoxy-9-phenanthrene carboxylic acid amide against HeLa cells with doxorubicin as a positive control.

| Sample | Concentration (ppm) | Average absorbance | % cell death | Probit | Log concentration | $IC_{50}$ (µg/ml) | Regression equation |
|---|---|---|---|---|---|---|---|
| Doxorubicin | 100<br>50<br>25<br>12,5 | 0,233<br>0,261<br>0,397<br>0,411 | 65,41<br>55,46<br>19,52<br>15,76 | 5,38<br>5,12<br>4,12<br>3,96 | 2,00<br>1,70<br>1,40<br>1,10 | 56,56 | y = 1,7533x + 1,9273<br>$R^2$ = 0,9157 |
| 10-(6,6-Dihydroxy-hexyl)-2,3,6-trimethoxy-phenanthrene-9-carboxylic acid amide | 75,04<br>37,52<br>18,76 | 0,128<br>0,463<br>0,616 | 94,25<br>34,89<br>7,87 | 6,57<br>4,58<br>3,52 | 1,87<br>1,57<br>1,27 | 39,05 | y = 5,0833x - 3,0908<br>$R^2$ = 0,9699 |

Based on Zakaria *et al.* (2012), the inhibition activity of sample is very strong when the $IC_{50}$ is <5 µg/ml; Strong is 5-10 µg /ml; moderate is 10-20 µg /ml, and weak when the $IC_{50}$ of sample is 20-100 µg / ml) and inactive (> 100 µg /ml), so that the compound 10- (6,6-dihydroxy-hexyl) -2,3,6-trimethoxy-phenanthrene-9-carboxylic acid amide have weak anti-proliferation activity against HeLa cells[15]. However, the $IC_{50}$ value of this compound is smaller than the positive control (doxorubicin, $IC_{50}$ 56.56 ug /ml) so that it can be said compound 10- (6,6-dihydroxy-hexyl) -2,3,6-trimethoxy-phenanthrene-9- carboxylic acid amide had a stronger inhibitory effect than doxorubicin.

Cell death caused by 10- (6,6-dihydroxy-hexyl) -2,3,6-trimethoxy-phenanthrene-9-carboxylic acid amide is not yet known with certainty. However, based on estimates of the proposed structure of the compounds, phenanthrene skeleton compounds is probably amongst one of the factors that give the activity of the compound. Rigidity phenanthrene is an important condition that the nitrogenous base can provide high cytotoxicity. As reported Stærk et al. (2002) conducted a study in vitro cytotoxic activity against drug sensitive some alkaloids KB-3-1 and multidrug-resistant cell line KB-VI, 13aR-antofine, 13aR-6-O-desmethylantofine and 13aR-7-O-desmethyltylophorine shows the $IC_{50}$ values between 7-17 nM while cytotoxicity effects seco analog-secoantofine 13aR and 13aR-6-Odesmethylsecoantofine with smaller rigidity drastically decreased (each have $IC_{50}$ values of 2630 and 403 nM)[16]. In addition, the proposed ring phenanthrene compound contained methoxy groups at C-2, the cluster is an important group that may provide cytotoxic activity of compounds 10- (6,6-dihydroxy-hexyl) -2,3,6-trimethoxy-phenanthrene- 9-carboxylic acid amide.

As studies conducted by Su et al. (2008), which saw a decrease of cytotoxicity (10-70 times) in deoxypergularinine compared antofine, Su et al. (2008) found that the absence of a methoxy group at C-2 as shown in deoxypergularinine can reduce the activity of the compound compared with antofine, which has methoxy groups at C-2 atom[17]. This indicates that the ring phenanthrene groups are essential in providing optimal cytotoxic activity. So based on some of the above analysis it can be concluded that the expected structure of the compound proposed theoretically can deliver anticancer activity as seen on the test result data antiproliferation against HeLa cells.

## CONCLUSION

One isolates obtained from the leaves of parang Romang (*B. virgata*) is BVI03, through $^{1}$H and +C NMR data is expected the structure of the compound is 10- (6, 6-dihydroxy-hexyl) -2,3,6-trimethoxy-phenanthrene-9-carboxylic acid amide. Through MTT test against HeLa cells are known this compound ($IC_{50}$ 39.05 ug/ml) had a stronger anticancer activity than doxorubicin ($IC_{50}$ 56.56 ug/ml).

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## REFERENCES

1. Desai AG, Qazi GN., Ganju RK, El-Tamer M, Singh J, Saxena AK, Bedi YS, Taneja SC, and Bhat HK, Medicinal Plants and Cancer Chemoprevention. *Current Drug Metab*olism, 9(7), 2008, 581–591.
2. Pusat Data dan Informasi Kementerian Kesehatan RI, Situasi Penyakit Kanker. Semester I, Buletin Jendela Data dan Informasi Kesehatan Republik Indonesia, 2(1), 2015, 2088 – 2091.
3. Nicolini A, Carpi A, Ferrari P, Biaya PM, Rossi G, Immunotherapy and Hormone-therapy in Metastatic Breast Cancer: A Review and Update, *Current Drug Targets,* 2016;17(10):1127-39.
4. Feinberg BA, Bruno AS, Haislip S, Gilmore J, Jain G, Whyte JL, Hemoglobin Trends and Anemia Treatment Resulting from Concomitant Chemotherapy in Community Oncology Clinics, *Journal of Oncology Practice*, 2012; 8(1): 18–23.
5. Manggau MA Hasan H, Wahyudin E, Haryono K, Mufidah, Muslimin L, Efek Farmakologi Tanaman Antikanker Yang Digunakan Oleh Masyarakat Suku Makassar Sulawesi Selatan, 3, Hasanuddin University Publisher, Makassar, 2012a, 90-94 .
6. Manggau MA, Yusriadi, Mufidah, Alam G, Antiproliferative effect of parang romang (*Boehmeria virgata* (Forst) Guill leaf extracts against HeLa cell line. *Majalah Farmasi dan Farmakologi*, 11(3), 2007, 76-79.
7. Wardihan, Rusdi M, Alam G, Lukman and Manggau MA, Selective Cytotoxicity Evaluation in Anticancer Drug Screening of *Boehmeria virgata* (Forst) Guill Leaves to Several Human Cell Lines: HeLa, WiDr, T47D and Vero. *Dhaka University Journal of Pharmaceutical Sciences*, 12(2), 2013, 87-90.
8. Manggau MA, Mufidah and Lindequist U, Antiproliferation Against Human Bladder Cancer 5637 Cell Line and Antioxidant Activity of Various Plant Extracts. *The Indonesian Journal of Natural Product*, 6, 2009, 247-250.
9. Manggau MA, Lukman, Rusdi M, Hatta M, Rahman L, and Subehan, Effect of an Isolated Active Compound (BVI03) of *Boehmeria virgata* (Forst) Guill Leaf on Antiproliferation in Human Cancer Cervix Hela Cells Through Activation of Caspase 3 and p53 Protein. *Majalah Farmasi dan Farmakologi*, 16(3), 2012b, 115 – 120.
10. Farnsworthn NR, Hart NK, Johns SR, Lamberton JA, and Messmer W, Alkaloids of *Boehmeria cylindrica* (famili urticaceae) : Identification of a cytotoxic agent, highly active Against eagle's 9kb carcinoma of the nasopharynx in cell culture, as crytopleurine. *Australian Journal of Chemistry*, 22, 1969, 1805-1807.
11. Cai XF, Jin X, Lee D, Yang YT, Lee K, Hong YS, Lee JH, and Lee JJ, Phenanthroquinolizidine Alkaloids from the Roots of *Boehmeria pannosa* Potently Inhibit Hypoxia-Inducible Factor-1 in AGS Human Gastric Cancer Cells. *Journal of Natural Products,* 69, 2006, 1095-1097.
12. Luo Y, Liu Y, Luo D, Gao X, Li B, Zhang G, Cytotoxic Alkaloid from *Boehmeria siemensis*. *Planta Medica Journal*, 69, 2003, 842-845.
13. Christodoulou MS, Calogero F, Baumann M, García-Argáez N, Pieraccini S, Sironi M, Dapiaggi F, Bucci R, Broggini G, Gazzola S, Liekens S, Silvani A, Lahtela-Kakkonen M, Martinet N, Nonel-Canals A, Santamaría-Navarro E, Baxendale IR, Dalla Via L, Passarella D, Boehmeriasin A as new lead compound for the inhibition of Topoisomerases and SIRT2. *European Journal of Medicinal Chemistry*, 92, 2015, 766-775.
14. Yan J, Luo D, Luo Y, Gao X, Zhang G, 2006. Induction of G1 arrest and differentiation in MDA-MB-231 breast cancer cell by boehmeriasin A, a novel compound from plant. *Int J Gynecol Cancer*, 16: 165–170.
15. Silverstein, Identification of Organic Compund. 3rd Edition, John Wiley & Sons Ltd, New York, 2002, 132-138.
16. Stærk D, Lykkeberg AK, Christensen J, Budnik BA, Abe F, and Jaroszewski JW, In Vitro Cytotoxic Activity of Phenanthroindolizidine Alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanakae* against Drug-Sensitive and Multidrug-Resistant Cancer Cells. *Journal of Natural Product,* 65, 2002, 1299-1302.

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