

Anticancer Activity of *Ruellia brittoniana* Flower on Cervical HeLa Cancer Cells

Nadzila Anindya Tejaputri¹, Ade Arsianti^{2,3,*}, Fona Qorina¹, Qotrunnada Fithrotunnisa¹, Norma Nur Azizah³, Rista Putrianingsih²

Nadzila Anindya Tejaputri¹,
Ade Arsianti^{2,3,*}, Fona Qorina¹,
Qotrunnada Fithrotunnisa¹,
Norma Nur Azizah³, Rista
Putrianingsih²

¹Medical Student, Faculty of Medicine
University of Indonesia, Depok, INDONESIA.

²Department of Medical Chemistry, Faculty
of Medicine, University of Indonesia, Depok,
INDONESIA.

³Drug Development Research Cluster, Drug
Discovery Division, Indonesia Medical
Education and Research Institute (IMERI),
Faculty of Medicine, University of Indonesia,
Jalan Salemba Raya 6 Jakarta 10430,
INDONESIA.

Correspondence

Dr. Ade Arsianti

Department of Medical Chemistry, Faculty
of Medicine, University of Indonesia,
Jalan Salemba Raya 6 Jakarta 10430,
INDONESIA.

Phone no. +6281312581253

Email: arsi_ade2002@yahoo.com

History

- Submission Date: 03-09-2019;
- Review completed: 09-10-2019;
- Accepted Date: 14-10-2019.

DOI : 10.5530/pj.2020.12.6

Article Available online

<http://www.phcogj.com/v12/i1>

Copyright

© 2020 Phcogj.Com. This is an open-
access article distributed under the terms
of the Creative Commons Attribution 4.0
International license.

ABSTRACT

Introduction: Cervical cancer ranks 4th in terms of the mortality rates and incidence of all cancers in women (GLOBOCAN 2018). In last decade, there is a significance progress in cancer therapy followed by an increase in the cost of cancer treatment. Therefore, it is necessary to have therapeutic innovations that are expected to reduce the cost of cervical cancer therapy. One therapeutic innovation that is currently being intensively carried out is herbal medicine. Some researchers have found that some plant extracts have anti-cancer properties that can be an alternative treatment for cancer, such as some plants with the genus *Ruellia*, such as *Ruellia tuberosa* and *Ruellia squarrosa*. However, research on the anticancer activity of the species of *Ruellia brittoniana*, especially the flowers, is still limited. **Objective:** Aim of this study is to examine anti-cervical cancer activity of *R. brittoniana* flower. **Methods:** *R. brittoniana* flowers were obtained from Depok, West Java, Indonesia. The flowers are extracted gradually with n-hexane, ethyl acetate, and ethanol solvents. The extracts were evaluated for anticancer activity by MTT method. **Result:** IC₅₀ values for ethanol extract, ethyl acetate extract and n-hexane extract of *R. brittoniana* flowers are 116.55 ppm, 52.62 ppm, and 123.09 ppm, respectively, which indicating that ethanol extract has moderate anticancer activity, while ethyl acetate and n-hexane extract of *R. brittoniana* flowers have weak anticancer activity. **Conclusion:** Ethanol, ethyl acetate, and n-hexane extract of *R. brittoniana* flowers have a potential to become natural anti-cervical cancer.

Key words: *Ruellia brittoniana*, Anticancer, Cervical HeLa cells.

INTRODUCTION

Cervical cancer is a malignancy that starts from cells lining the cervix. Based on GLOBOCAN 2018, cervical cancer ranks 4th in terms of the mortality rates and incidence of all cancers in women. Every year, cervical cancer causes death in 260,000 women and nearly 85% of these deaths occur in developing countries. In last decade, there is a significance progress in cancer therapy. Unfortunately, with advances in cancer treatment, there has also been an increase in the cost of cancer treatment. The high cost of cancer treatment has led to an increase in medical bankruptcy. In addition, cancer patients have twice the risk of experiencing bankruptcy than people who do not have cancer.¹⁻³

Therefore, it is necessary to have therapeutic innovations that are expected to reduce the cost of cervical cancer therapy. One therapeutic innovation that is currently being intensively carried out is herbal medicine. Herbal medicines are believed to be more natural and therefore do not have significant side effects and tend not to cause dependence. Some researchers have found that some plant extracts have anti-cancer properties that can be an alternative treatment for cancer, such as some plants of the genus *Ruellia*, such as *Ruellia tuberosa* and *Ruellia squarrosa*.⁴⁻⁷

In contrast, there is no previous researcher that reported the anticancer activity of *Ruellia*

brittoniana flower. This fact prompted us to explore anticancer activity of *R. brittoniana* against cervical HeLa cells. In extraction method, the use of solvents that have a variety of polarity should be done to ensure that various compounds with different polarity can be extracted properly. Therefore, in this work, we conducted extraction of *R. brittoniana* flower in polar solvent of ethanol, semipolar solvent of ethyl acetate, and non-polar solvent of n-hexane, respectively.^{8,9}

METHODS

Extraction of *Ruellia brittoniana* flowers

R. brittoniana flowers were dried and ground to give a powder. The powder then macerated gradually three times with n-hexane, ethyl acetate, and ethanol solvent. Maceration was done by soaking the powder of *R. brittoniana* in 500 mL of solvent for 24 hours and then filtrated. Filtrate were collected, whereas the solid is used for further maceration. Collected extract was then dried by vacuum rotary evaporator to produce crude extract of *R. brittoniana* flower.¹⁰

MTT method

Anticancer activity of the extracts of *R. brittoniana* flowers were evaluated by the MTT method. *R. brittoniana* flowers extracts were dissolved in *Dimethyl Sulfoxide* (DMSO) and then added to HeLa

Cite this article: Tejaputri NA, Arsianti A, Qorina F, Fithrotunnisa Q, Azizah NN, Putrianingsih R. Anticancer activity of *Ruellia brittoniana* on HeLa Cells. *Pharmacogn J.* 2020;12(1):29-34.

cervical cancer cell. After that, samples were incubated in CO₂ incubator for 24 hours. MTT reagent with concentration 5 mg / mL in phosphate-buffered saline were added to samples and re-incubated during 4 hours. Subsequently, the DMSO solution was added to dissolve bluish purple deposits in the samples. In the final step, the absorbance of the solution was measured using an ELISA Reader.¹¹

The absorbance of the solution was then calculated the rate of inhibition using formula:¹¹

$$\% \text{Inhibition} = 1 - \left(\frac{\text{absorbance of treatment group}}{\text{absorbance of control group}} \right) \times 100\%$$

Absorbance of control group = Absorbance from HeLa cervical cancer cells and complete medium

Absorbance of treatment group = Absorbance from HeLa cervical cancer cells and extract of *R. brittoniana* flower

Statistical analysis

The IC₅₀ value obtained in this research was obtained from linear regression analysis in the Microsoft Excel Program.

RESULTS

Extraction samples of *Ruellia brittoniana* flowers

In this study, the extraction process was produced three extracts, i.e. ethanol extracts, ethyl acetate extracts, and n-hexane extracts of *R. brittoniana* flower.

MTT method

The results of the cytotoxic activity test of ethanol extract of *R. brittoniana* flowers on HeLa cervical cancer cells are presented in Figure 1. The graph in Figure 1 shows that there is a positive relationship between the log concentration of ethanol extract and percentage of inhibition on HeLa cervical cancer cell, it means that the higher log concentration, the higher percentage of inhibition on HeLa cells (Figure 2). Therefore, the largest percentage of inhibition, ie 67.8%, occurs in administration of a maximum ethanol extract concentration of 200 ppm. The linear regression equation from the graph is $y = 42.148x - 37.099$. The antilog value of the x coefficient is proportional to the IC₅₀ value, which is the concentration needed to inhibit 50% growth of HeLa cervical cancer cells. Based on the calculation of the linear regression equation, the IC₅₀ value of ethanol extract was 116.55 ppm.

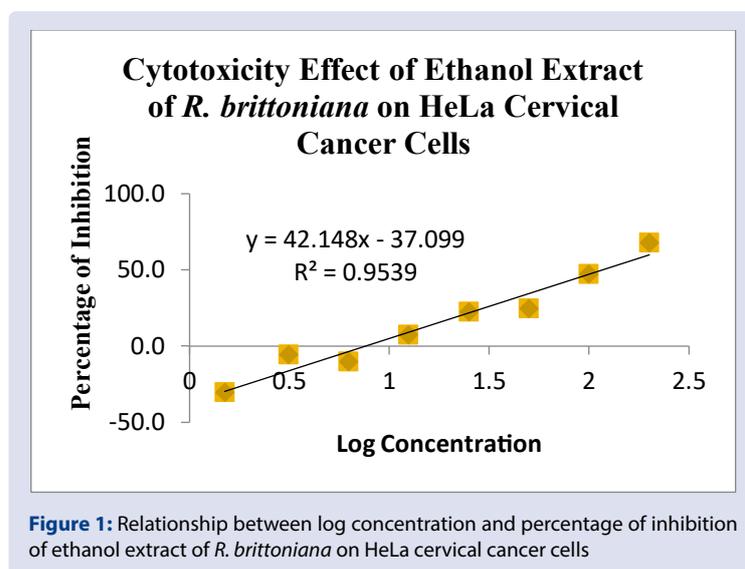
Anticancer activity evaluation of ethyl acetate extract of *R. brittoniana* flowers on HeLa cervical cancer cells are presented in Figure 3. The graph in Figure 1 shows that the higher log concentration of ethyl acetate extract of *R. brittoniana* flower, the higher percentage of inhibition on HeLa cells. Percentage of inhibition increased from -7.0% until 67.9% as concentration increases. The linear regression equation from the graph is $y = 39.346x - 17.72$. Based on the calculation through linear regression equation, the IC₅₀ value of ethanol extract is 52.62 ppm. Anticancer activity of n-hexane extract of *R. brittoniana* flowers on HeLa cervical cancer cells are presented in Figure 4. The graph in Figure 3 shows that percentage of inhibition on HeLa cervical cancer cell would be higher as the log concentration increases. The highest percentage of inhibition is 68,9%, which is occurs in administration of a maximum concentration of n-hexane extract at 200 ppm. The linear regression equation from the graph is $y = 35.727x - 24.678$. Based on the calculation through the linear regression equation, the IC₅₀ value of ethanol extract is 123.09 ppm.

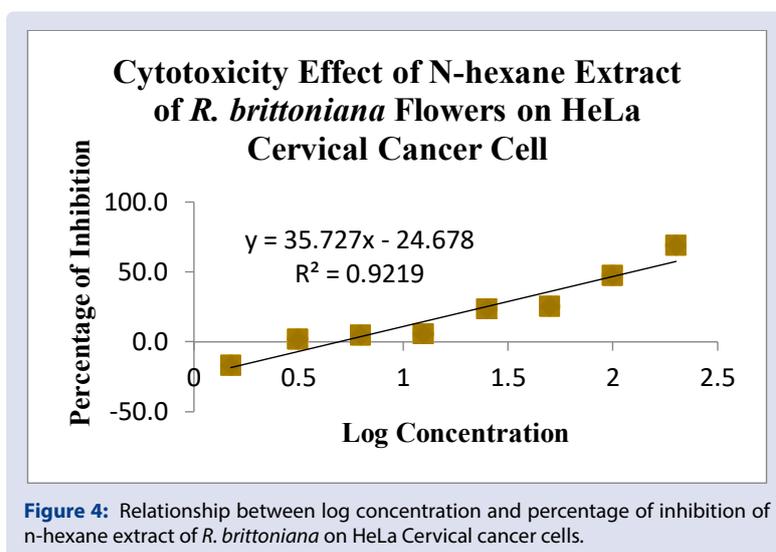
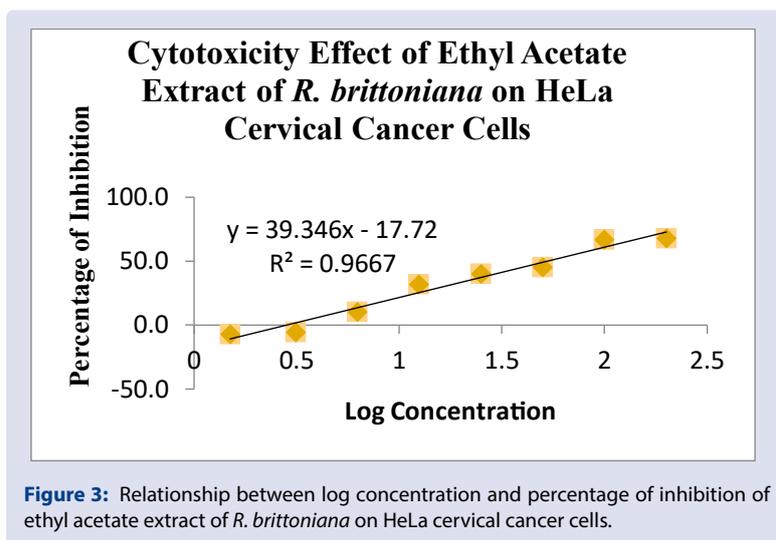
Among three extracts of *R. brittoniana* flowers, ethyl acetate extract has the smallest IC₅₀ value. This suggesting that ethyl acetate extract has the strongest anticancer activity against HeLa cells compared to ethanol and hexane extracts. Meanwhile, n-hexane extract has the largest IC₅₀ value, which indicating that n-hexane extract has lowest ability to inhibit a growth of HeLa cells. In this study, cisplatin as a positive control, has an IC₅₀ value of 1.78 ppm. Compared to extracts of *R. brittoniana* flowers, cisplatin has a smaller IC₅₀ value, this shows that the three extracts of *R. brittoniana* have a weaker ability to inhibit the growth of HeLa cells compared to cisplatin.

DISCUSSION

Extraction

In this study, the extraction process of *R. brittoniana* flowers is carried out in a variety of solvents, from n-hexane with low polarity solvent of n-hexane and semi-polar solvent of ethyl acetate to high polarity solvent of ethanol, which is required to ensure that phytochemical compound with various polarity can be extracted well in suitable solvents. Polar solvent such as ethanol can extract well alkaloids, saponins, flavonoids, and polyphenol. While semi-polar solvent such as ethyl acetate can be used to extract alkaloids, flavonoids, and triterpenoids. Non-polar solvent such as n-hexane can extract well lipophilic chemical compounds, such as triterpenoids and alkaloids.^{8,9}





MTT method

The principle of MTT method is the reduction of the yellow salt tetrazolium MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by the reductase system in respiration chain in the mitochondria of living cell which will form purple water-insoluble formazan crystals. The addition of DMSO will dissolve the purple crystal which is then absorbed through the ELISA reader. The intensity of the resulting purple color illustrates the large number of living cells. If the intensity of the purple color is higher, the more number of living cells.¹¹

Based on the graphs in Figures 1,2 and 3, it can be observed that there is a positive correlation between percent inhibition of HeLa cervical cancer cell activity with log concentration of *R. brittoniana* flower extract, ie the higher concentration of *R. brittoniana* flower extract given, the higher the percent inhibition of cervical cancer cells HeLa. Besides being able to see the relationship between percent inhibition and extract concentration, linear equation can be obtained from graph that will be used in calculating IC₅₀ values. IC₅₀ value is the concentration of the extract needed to inhibit 50% of HeLa cervical cancer cell activity. Based on calculations, IC₅₀ values for ethanol extract, ethyl acetate extract and n-hexane extract are 116.55 ppm, 52.62 ppm, and 123.09 ppm, respectively. Meanwhile, IC₅₀ value for cisplatin as positive control is 1.78 ppm.¹¹

Atjanasuppat et al classified the anticancer activity of a compound based on IC₅₀ values. Compound with an IC₅₀ value <20 ppm is classified as compound with strong anticancer activity, compound with an IC₅₀ value >20-100 ppm is a compound with moderate anticancer activity, compound with IC₅₀ value >100-1000 is classified as compound with weak anticancer activity, and compound with IC₅₀ value >1000 is a compound that inactive as anticancer. Based on the classification, ethanol and n-hexane extract of *R. brittoniana* flower which have an IC₅₀ value >100 ppm are classified as compounds with weak anticancer activity. Meanwhile, ethyl acetate extract of *R. brittoniana* flower which has an IC₅₀ value in range of >20-100 ppm is classified as compound with moderate anticancer activity. Cisplatin as positive control classified as compound with strong anticancer activity. The comparison between three extracts of *R. brittoniana*, extract of *R. brittoniana* flowers that have the strongest anticancer activity is ethyl acetate extract and extract of *R. brittoniana* flowers that have the weakest anticancer activity is n-hexane extract. The difference in IC₅₀ values of the three *R. brittoniana* flowers extracts is influenced by the various polarity of the solvent in the extraction process which affects the phytochemical content of each extract. Different phytochemical content will affect the results of different cytotoxic activities.^{8,9,12}

No previous studies have been found that report the anticancer activity of *R. brittoniana* flowers on cancer cell lines. However, there are some previous studies that report the cytotoxicity of other species in the genus *Ruellia* against cancer cells, such as *Ruellia tuberosa*. Based on studies by Afzal et al, methanol extracts of *Ruellia tuberosa* have cytotoxic properties with IC₅₀ values of 3.5 and 1.9 ppm on H460 and MDAMB231 cancer cells, respectively. Studies by Samy MN et al. reported that phytochemical compound of cirsimaritin and cirsimaritin in *Ruellia tuberosa* show in vitro cytotoxic activity against KB cell line at doses of 30.05 and 17.91 µg / ml.^{6,7,13,14}

CONCLUSION

Ethanol, ethyl acetate, and n-hexane extracts of *R. brittoniana* flowers have a potential to be developed as natural anti-cervical cancer agent. Further studies are needed to evaluate the anticancer effects by *R. brittoniana* flower on a molecular basis.

ACKNOWLEDGEMENT

We express our appreciation to Directorate of research and Public Service University of Indonesia for PITTA B (Publikasi International Terindeks Scopus Untuk Tugas Akhir Mahasiswa B) research grant for fiscal year 2019.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

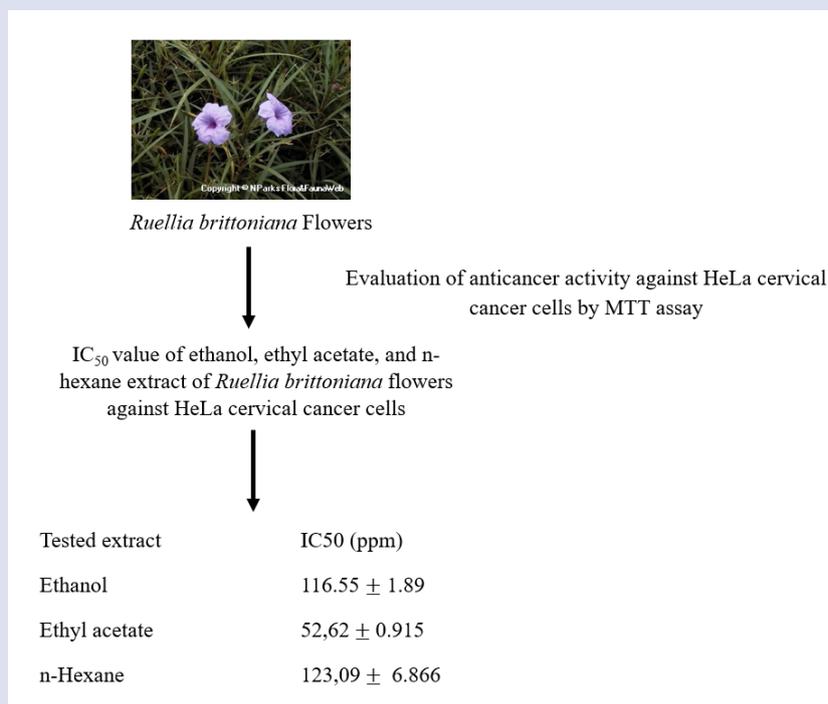
ABBREVIATIONS

DMSO: *Dimethyl Sulfoxide*; HeLa: Henrietta Lack; IC₅₀: Inhibition Concentration 50%; %: Percentage; MTT:(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); *R. brittoniana*: *Ruellia brittoniana*.

REFERENCES

1. Komite penanggulangan kanker nasional. Panduan penatalaksanaan kanker serviks. Jakarta: Kementerian kesehatan republik Indonesia; 2017:36.
2. World Health Organization. Latest global cancer data: cancer burden rises to 18. 1 million new cases and 9.6 million cancer deaths in 2018 [Internet]. France: WHO; 2018 [cited 2019 August 9]. Available from: <https://www.iarc.fr/wp->
3. Kelly RJ, Forde OM, Elnahal SM, Forastiere AA, Rosner GL, Smith TJ. Patients and physicians can discuss costs of cancer treatment in the clinic. *J Oncol Pract.* 2015;11(4):308-12.
4. Tavakoli J, Miar S, Zadehzare MM, Akbari H. Evaluation of effectiveness of herbal medication in cancer care: a review study. *Iran J Cancer Prev.* 2012;5(3):144-56
5. Karimi A, Majlesi M, Kopaei MR. Herbal versus synthetic drugs; beliefs and facts. *J Nephropharmacol.* 2015;4(1):27-30.
6. Afzal K, Uzair M, Chaudhary BA. Genus ruellia: pharmacological and phytochemical importance in ethnopharmacology. *Acta Poloniae Pharmaceutica Drug Research.* 2015;72(5):821-7.
7. Afzal MK, Uzair M, Chaudhry BA. Anticancer activity of Ruelliasquarrosaaagaints human prostate cancer cell line. *Bangladesh J Pharmacol.* 2015;10:97-99.
8. Jadid N, Hidayati D, Hartanti SR, Arraniry BA, Rachman RY, Wikanta W. Antioxidant activity of different solvent extracts of Piper retrofractum Vahl. using DPPH assay. *AIP Conf Proc.* 2017;1854(1):020019-1-5.
9. Banu KS, Cathrine L. General techniques involved in phytochemical analysis. *International Journal of Advanced Research in Chemical Science (IJARCS).* 2015;2(4):25-32.
10. Minarno EB, Skiringfitokimia dan kandungan total flavonoid pada buah Caricapubescens lenne & k. koch di kawasan bromo, cangar, dan datarantinggi dieng. *El-Hayah.* 2015;5(2):73-82.
11. Putri H. Protokol uji sitotoksik metode MTT. *Jogja: Cancer chemoprevention research center fakultas farmasi UGM*; 2013. Available from: <http://ccrc.farmasi.ugm.ac.id/wp-content/uploads/03.010.02-uji-sitotoksik-MTT.pdf>.
12. Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A. In vitro screening for anthelmintic and antitumour activity of ethnomedicinal plants from Thailand. *J Ethnopharmacol.* 2009;123:475-82.
13. Samy MN, Sugimoto S, Mastunami K, Otsuka H, Kamel MS. Chemical constituents and biological activities of genus *Ruellia*. *IJP.* 2015;2(6):270-9.
14. *Ruellia simplex* C. Wright [internet]. Singapore: National parks board; 2013 [cited at 2018 Jul 1]. Available from: <https://florafaunaweb.nparks.gov.sg/special-pages/plant-detail.aspx?id=2397>

GRAPHICAL ABSTRACT



SUMMARY

R. brittoniana shows cytotoxicity activity against HeLa cells, with IC₅₀ values for ethanol extract, ethyl acetate extract and n-hexane were 116.55 ± 1.89 ppm, 52.62 ± 0.915 ppm, and 123.09 ± 6.866 ppm, respectively.

ABOUT AUTHORS



Dr. Ade Arsianti: Lecture and Researcher at Medical Chemistry and Drug Development Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia. Research interest medicinal chemistry, Synthetic Organic Chemistry and natural product chemistry.



Nadzila Anindya Tejaputri: Medical Student, Faculty of Medicine, Universitas Indonesia. Research interest in herbal medicine, cancer biology, pediatric disease, and mental health science.



Qotrunnada Fithrotunnisa: Medical Student, Faculty of Medicine, Universitas Indonesia. Research interest in herbal medicine and cancer biology



Norma Nur Azizah: Researcher at Drug Development Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia. Research interest tissue culture, analytical chemistry, and natural product chemistry in drug development.



Rista Putrianingsih: Researcher at Medical Chemistry, Faculty of Medicine, Universitas Indonesia. Research interest tissue culture and natural product chemistry.

Cite this article: Tejaputri NA, Arsianti A, Qorina F, Fithrotunnisa Q, Azizah NN, Putrianingsih R. Anticancer activity of *Ruellia britoniana* on HeLa Cells. *Pharmacog J.* 2020;12(1):29-34.