**O (Oral)**

**THE CYTOTOXIC EFFECT OF FOUR FRACTIONS FROM PURPLE NUTSEDGE (*Cyperus rotundus* L.) TUBER ESSENTIAL**

**OIL ON THE HELA CELL**

***Susianti Susianti*1\*, Yanwirasti Yanwirasti2, Eryati Darwin3, Jamsari Jamsari4**

1Department of Histology, Faculty of Medicine, Lampung University, Bandar lampung, Indonesia

2Department of Anatomy, Faculty of Medicine, Andalas University, Padang, Indonesia

3Department of Histology, Faculty of Medicine, Andalas University, Padang, Indonesia

4Faculty of Agriculture, Andalas University, Padang, Indonesia

\*Corresponding author.

Email: susiantiglb@yahoo.com

**ABSTRACT**

**Objective:** The aim of this research is to investigate the cytotoxic effect of four fractions from purple nutsedge tuber essential oil on HeLa cervical cancer cell line .

**Methods:** The method used in this research was a cytotoxic test on HeLa cervical cancer cell line using an MTT assay. The cells were incubated along 24 hours with four fractions of purple nutsedge essential oil in a 96-well plate with eight series of doses (0.625-80μg/ml) for each fraction. Each dose was performed three times. After the absorbance of the cells was measured using an ELISA reader, the percentage of cell viability was calculated for each dose, followed by the calculation of the inhibitory concentration 50% rate (IC50) mean using probit regression analysis.

**Results:** The results of this research showed that fraction 1 IC50 (mean+standard deviation) was 8.307 + 0.186 μg/ml, fraction 2 was 21.377 + 9.543 μg/ml, fraction 3 was 1,707.521+1,048.319µg/ml and fraction 4 was 4,398.836+3,476.323 µg/ml. It means that fraction 1 and 2 have strong cytotoxic effect and fraction 3 and 4 have not cytotoxic effect on HeLa cervical cancer cell line.

**Conclusion**: The conclusions of this research are there was cytotoxic effect of fraction 1 and 2 from purple nutsedge tuber essential oil fraction on HeLa cervical cancer cell line.

**Keywords**: *Cyperus rotundus* L., Purple Nutsedge, Essential Oil, Fraction, Cervical Cancer

**INTRODUCTION**

 There are many drug of cancer have been used in chemotherapy. The drugs often used include antimetabolites, DNA-interactive agents, antitubulin substances, hormones, and other substances that have molecular targets. However, the use of chemotherapy drugs often leads to undesirable effects, such as hair loss, bone marrow suppression, drug resistance, gastrointestinal system damage, neurological dysfunction, and cardiac toxicity1. For this reason there are many research have been done to obtain natural materials for anticancer agent from the plant both *in vitro* and *in vivo*. The mechanism of them are reducing proliferation, inducing apoptosis, slowing metastasis, and inhibiting angiogenesis1,2.

 Purple nutsedge (*Cyperus rotundus* L.) is one of the medicinal plants that has the potential to be developed as an anticancer substance. This plant is potential to develop because it is cheap and easy to obtain. It has long been used as a remedy for various diseases, such as diarrhea, inflammation, diabetes, fungus, and cancer; has antimicrobial, antioxidant, antimutagenic, antipyretic, analgesic, anti-emetic, and anti-obesity effects; and can be used as a stimulant, diuretic, and sedative3,4,5. A variety of studies have been done on the purple nutsedge tuber as an anticancer substance. We hope that the material has higher efficacy and more minimal side effects1,6.

 Purple nutsedge is widespread and grows in South Africa, Korea, China, Japan, Taiwan, Malaysia, Indonesia, and Southeast Asia. It grows on unusually dry farmland, in fields, and in gardens7. Purple nutsedge has different name for different locations. In Arabic it is called *Saed, Sajal* and *Seil*. In English it is often called *nut grass*, *purple nutsedge*, or *Nagarmotha*; in China it is called *Xiang Fu* (Al-Jumaily et al., 2014); and in Indonesia it is called *rumput teki*. A part of the purble nutsedge often used is the tuber. It is shaped like a little finger and can be round or oval and wrinkled or grooved. It feels a bit prickly and the outside is brown while the inside is white, similar to spices; it tastes bitter7,8. A variety of studies have been done on the purple nutsedge tuber as an anticancer substance.

 From various studies have identified various chemical compounds in the purple nutsedge tuber in the form of antioxidants and other compounds that are suspected to have medical effects and potential to be developed as a drug. Purple nutsedge tuber contents include alkaloids, flavonoids, glycosides, furochromones, monoterpenoids, sesquiterpenoids, tannins, sitosterols, fats, polyphenols and essential oils5,9,10. Essential oils have been widely studied to have anticancer effects as both an antioxidant and also trigger apoptosis. The induction of apoptosis by volatile oil can be through various mechanisms, including through p53 as well as by increasing Bax protein and decreasing Bcl-2 protein6.

 In developing anticancer drugs derived from natural ingredients is not enough only to the level of extract, but must be traced to active compounds contained in a plant. In the preliminary study we can do fractination of the material from plants. In this research, we will make fractination of purple nutsedge essential oil and then cytotoxic test to servical cancr cell line HeLa.

**METHODS**

**Chemical and reagents**

 The purple nutsedge tubers used in this study came from the wild areas surrounding Bandar Lampung City, Lampung Province, Indonesia. The initial process in this research was to identify and determine which plants would be used based on the observation of plant physiological characteristics such as flowers, leaves, stems, roots, and tubers. The next step was to ensure the true convinced purple nutsedge (*C. rotundus* L.) by using the material test determination in the Botanical Laboratory in the Biology Department of Mathematics and Natural Sciences Faculty at Lampung University. After this determination was done, several stages of material test preparation process was performed, namely taking essential oil from the purple nutsedge tubers through the process of steam distillation. The purple nutsedge tubers were washed and then dried at room temperature for about one week, after which they were cut into small sizes. A total of 10kg of dry tubers was distilled with aqua 2/3 of pumpkin contents for approximately 4 hours. Furthermore, the essential oil, which was still mixed with a little water, was removed by adding MgSO4 7H2O until the liquid was saturated. A total of 15 ml of volatile oil was produced by the steam distillation process and then stored in dark and closed glass bottles.

 In the fractionation process the essential oil of grass teki 8 g was inserted in a 50 g chromatography column gel silica and eluted with 3% MeOH / CHCl3 of 1 L. The eluate was then collected in 10 mL of each reaction tube. The eluates in the test tube were then analyzed by Thin Layer Chromatography (TLC) on the silica gel plate. The TLC plates were eluted in a chamber containing a 3% MeOH / CHCl3 developer solution. The TLC plate is then dried and the spot profile is visible under UV lamp. From 8g of essential oil obtained 4 fractions with the details of fraction 1 as much as 0.967g, fraction 2 5.291g, fraction 3 0.832g, and fraction 4 0.237g.

**Cytotoxicity Test with MTT Assay**

 Based on Mosman11, the cytotoxic test was assessed by using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) dye reduction assay. It was performed using a 96-well microculture plate. Each well in the plate was filled with a HeLa cell suspension of 2 x 104 cells dissolved in 100 μl culture medium (RPMI 1640) containing 0.5% FBS (fetal bovine serum). The cells were then incubated (starvation) for 24 hours in a 5% CO2 incubator at 37°C. After incubation, the media in each well was removed, then replaced with new media containing 10% FBS and treatment with material test (each fraction of purple nutsedge tuber essential oil) was done in 8 serial doses (80; 40; 20; 10; 5; 2.5; 1.25; 0.625 μg/ml) and doxorubicin (positive control) in 8 serial doses (80; 40; 20; 10; 5; 2.5; 1.25; 0.625 μg/ml). Each dose was performed three times. The microculture plate was then incubated for 24 hours in a 5% CO2 incubator at 37°C. After that the media was removed and 100 μl of new medium and 10 μl MTT solution were added to each well and then incubated for 4 hours in a 5% CO2 incubator at 37°C. After that, 100 μl of sodium dodecyl sulfate (SDS) was added amounted 10% in 0.01% HCl and then the microplate was shaken at room temperature for 5 minutes, wrapped with aluminum foil, and incubated at room temperature overnight. The microplate was then read for absorbance using an ELISA reader at 595 nm wavelength. The percentage of living cells for each repetition (cell viability) was obtained by the formula:

(A-B) x 100%

(C-B)

  A = Average absorbance of media + cell + test material

  B = Average media absorbance

  C = Average absorbance of media + cell

**Ethics Approval**

 This research was experimental research using a human cervical cancer cell line (HeLa), so ethics approval was needed. Ethical clearance for this research was approved by the Research Ethics Committee of the Faculty of Medicine, Lampung University N0. 228/ UN26/ 8/ DT/ 2016, dated January 28, 2016.

**Statistical Analysis**

 The percentage of cell viability of each test material was converted into a dose-response curve using probit analysis, and then inhibitory concentration IC50, which is the concentration of each test material that causes the number of living cells about 50%, was obtained.

**RESULTS**

 The cytotoxic activity of the each fraction from purple nutsedge essential oil as shown by a dose-response curve can be seen in Figure 1. The table clearly shows that by increasing the concentration of essential oils provided to the HeLa cells, the viability of the HeLa cells decreases. This shows that the essential oils have cytotoxic activity in the HeLa cells. From the percentage of cell viability, the inhibitory concentration (IC50) can be calculated using probit regression analysis, and from this research we know that IC50 of fraction 1 is 8.307 + 0.186 μg / ml, fraction 2 is 21.377 + 9.543 μg / ml, fraction 3 is 1,707.521+1,048.319µg/ml and fraction 4 is 4,398.836+3,476.323µg/ml. The IC50 of doxorubicin to HeLa cells is 5.588 + 0.490 μg/ml. The comparative curve between the all fraction of purple nutsedge essential oils and doxorubicin is illustrated in Figure 1. The curve shows that increasing the concentration of the purple nutsedge doses can decrease the viability of HeLa cells.

Figure 1. Comparison of cytotoxic activity of the test material

**DISCUSSION**

 Based on the standards of the National Cancer Institute (NCI) in the United States, an extract has quite a lot of potential to be developed as an anticancer agent if it has 50% inhibitory concentration (IC50) < 50 μg/ml (Mans et al., 2000). If a compound has IC50 > 100 μg/ml, then it has a weak cytotoxic effect, whereas if it has IC50 > 400 μg/ml, it is not toxic12. This means that the fraction 1 and 2 of essential oils tested in this study have a strong cytotoxic and then fraction 3 and 4 are not toxic. This result does not differ from other research where purple nutsedge tuber essential oil was investigated and found to have a very strong cytotoxic effect on murine lymphoblastic leukemia (L1210) cells. However, that research did not provide information about IC50 13.

 In some studies, purple nutsedge was shown as having cytotoxic effects on cancer cells, thus revealing its potential for development as an anticancer agent. The methanol extract of the purple nutsedge stem has been found to have a weak cytotoxic effect on leukemia cell K562 and in L1210 cells through the induction of apoptosis in L121010. Sayed et al.14 proved that steroid glycosides from the purple nutsedge stem have a cytotoxic effect on mouse lymphoma cells (L5178Y). Kilani et al.13,15 tested purple nutsedge tuber extract on leukemia cells (L1210) and found that the extract has a cytotoxic effect by inducing apoptosis. Research that isolated the essential oils contained within the purple nutsedge also found the same effect. Chloroform and methanolic extracts of purple nutsedge tuber have also been found to have cytotoxic effects on HeLa and SiHa cervical cancer cells through apoptotic mechanisms. The cytotoxic effect of chloroform extract was stronger than the methanol extract3.

 Most essential oils were initially identified and used for the treatment of inflammatory and oxidative diseases. But in the development of purple nutsedge, its research as an anticancer substance continued because there is a relationship between the production of reactive oxygen species with the origins of oxidation and inflammation that can cause cancer. A variety of studies have identified various compounds in purple nutsedge in the form of antioxidants and variuous compounds that are suspected to have medical effects and the potential to be developed as drugs. Purple nutsedge contains alkaloids, flavonoids, glycosides, furochromones, monoterpenes, sesquiterpenes, tannins, sitosterol, fats, polyphenols, and essential oils5,9,10. Essential oils have been widely studied to have anticancer effects as both antioxidants and triggers of apoptosis. The induction of apoptosis by essential oils can occur through various mechanisms, including through p53, increasing Bax protein, and decreasing Bcl-2 protein6. The main essential oil compounds that have been isolated from purple nutsedge are α-Cyperone, cyperene, cyperotundone, cyperol, β-selinene, β-caryophyllene, valerenal, sugeonyl acetate, α-copaene, patchhoulene, trans-pinocarveol, patchoulenenone, aristrol-9-en-3-one, selina-4, 11 diene, aristrol-9-en-8-one, kobusone, sugetriol, isokobusone, isocyperol, sugeonol, and sitosterol5. Differences in the soil conditions, climate, and environment where purple nutsedge grows will cause differences in the composition of its essential oils. In a study that compared purple nutsedge from different parts of Africa, the same primary compounds of cyperene and α-Cyperone16 were obtained. However, the essential oils from C. *rotundus* obtained from the Riyadh region revealed some variations in the composition and percentage of their compounds when compa red with other C. *rotundus* essential oils from different areas around the world17. Based on Chen et al.’s research (2011), the main components of the essential oil of purple nutsedge tubers are cyperene (41.03%), β-caryophyllene oxide (5.32%), *α*-selinene (4.37%), *α-*copaene (4.36%), naphthalene, 6-isoproenyl-4, 8a-dimethyl-1, 2, 3, 5, 6, 7, 8, 8a-octahydro- (3.80%), and *α-*Cyperone (3.11%). From several researchs above, the composition of essential oil of the purple nutsedge tuber in different place is not same. So, the further investigation is need.

**CONCLUSION**

The conclusions of this research are there was cytotoxic effect of fraction 1 and 2 from purple nutsedge tuber essential oil fraction on HeLa cervical cancer cell line.

**ACKNOWLEDGEMENT**

This study is supported by Medical Faculty of Lampung University, Indonesia

**REFERENCES**

1. Hosseini, A.G., Cancer therapy with phytochemicals: Evidence from clinical studies. AJP 5(2): 84–97 (2015).
2. Galati, G., P.J. O’Brien, Potential toxicity of flavonoids and other dietary phenolics: Significance for the chemopreventive and anticancer properties. *Free Radic Biol Med* 37(3): 287–303 (2004).
3. Susianti, Selektivitas ekstrak umbi rumput teki (*Cyperus rotundus* L.) terhadap sel HeLa dan SiHa serta pengaruhnya terhadap apoptosis. *Thesis,* Universitas Gadjah Mada, Yogyakarta (2009).
4. Sivapalan, S.R., Medicinal uses and pharmacological activities of *Cyperus rotundus* Linn—A review. *International Journal of Scientific and Research Publications* 3(4): 1–8 (2013).
5. Singh, N., B.R. Pandey, P. Verma, M. Bhalla, M. Gilca, Phyto-pharmacotherapeutics of *Cyperus rotundus* Linn. (Motha): An overview. *468 Indian J Nat Prod Resour* 3: 467–476 (2012).
6. Gautam, N., A.K. Mantha, S. Mittal, Essential oils and their constituents as anticancer agents: A mechanistic view. *BioMed Research International*: 1–23 (2014).
7. Sudarsono, A. Pudjoarinto, D. Gunawan, S. Wahyuono, I.A. Donatus, M. Dradjad, et al., *Tumbuhan Obat.* Yogyakarta: Pusat Penelitian Obat Tradisional Universitas Gadjah Mada (PPOT-UGM), pp: 72–76 (1996).
8. Anonymous, *Inventaris Tanaman Obat Indonesia (I).* Vol 1. Departemen Kesehatan dan Kesejahteraan Sosial RI, Badan Penelitian dan Pengembangan Kesehatan, Jakarta (2000).
9. Zhou, Z., W. Yin, Two novel phenolic compounds from the rhizomes of *Cyperus rotundus*. *Molecules* 17(11): 12636–12641 (2012).
10. Soumaya, K.J., G. Zied, N. Nouha, K. Mounira, G. Kamel, H.D.M. Genvi, et al., Evaluation of in vitro antioxidant and apoptotic activities of *Cyperus rotundus*. *Asian Pacific Journal of Tropical Medicine* 7(2):105–112 (2014).
11. Mosman, T., Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65: 55–63 (1983).
12. Mathabe, M.C., A.A. Hussein, R.V. Nikolova, A.E. Basson, J.J.M. Meyer, N. Lall, Antibacterial activities and cytotoxicity of terpenoids isolated from Spirostachys Africana*. J Ethnopharmacol* 116: 194–197 (2008).
13. Kilani, S., J. Ledauphin, I. Bouhlel, S.M. Ben, J. Boubaker, I. Skandrani, et al., Comparative study of *Cyperus rotundus* essential oil by a modified GC/MS analysis method: Evaluation of its antioxidant, cytotoxic, and apoptotic effects. *Chem Biodivers* 5: 729–742 (2008a).
14. Sayed, H.M., M.H. Mohamed, S.F. Farag, G.A. Mohamed, P. Proksch, A new steroid glycoside and furochromones from *Cyperus rotundus* L. *Nat Prod Res* 21(4): 343–350 (2007).
15. Kilani, S., B.M. Sghaier, I. Limem, I. Bouhlel, J. Boubaker, W. Bhouri, et al., *In vitro* evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of *Cyperus rotundus. Bioresour Technol* 99: 9004–9008 (2008b).
16. Lawal OA, Oyedeji AO, 2009. Chemical composition of the essential oils of *Cyperus rotundus* L. from South Africa. *Molecules* 14(8)*:* 2909-2917
17. Al-Massarani, S., F. Al-Enzi, M. Al-Tamimi, N. Al-Jomaiah, R. Al-amri, K.H.C. Baser, et al., Composition & biological activity of *Cyperus rotundus* L. tuber volatiles from Saudi Arabia. *Nat. Volatiles & Essent. Oils* 3(2): 26–34 (2016).