Pak. J. Biotechnol. Vol. 15 (1) 77-81 (2018)
www.pjbt.org

ISSN Print: 1812-1837
ISSN Online: 2312-7791

THE CYTOTOXIC EFFECTS OF PURPLE NUTSEDGE (*Cyperus rotundus* L.) TUBER ESSENTIAL OIL ON THE HELA CERVICAL CANCER CELL LINE

Susianti Susianti[1], Yanwirasti Yanwirasti[2], Eryati Darwin[3], Jamsari Jamsari[4]

[1]Department of Histology, Faculty of Medicine, Lampung University, Bandar lampung, Indonesia. [2]Department of Anatomy, Faculty of Medicine, Andalas University, Padang, Indonesia. [3]Department of Histology, Faculty of Medicine, Andalas University, Padang, Indonesia. [4]Faculty of Agriculture, Andalas University, Padang, Indonesia
Email: susiantiglb@yahoo.com

Article received 22.8.2017, Revised 10.1.2018, Accepted 18.1.2018

ABSTRACT

Many problems in cancer therapy have prompted researchers to explore natural materials to discover new anticancer substances with higher efficacy and more minimal side effects. Purple nutsedge (*Cyperus rotundus* L.) is one of the potential medicinal plants being studied as an anticancer substance. A variety of studies have been conducted on the use of the purple nutsedge tuber as an anticancer substance, but not about the effects of its essential oils on cervical cancer. The method used in this research was a cytotoxic test on HeLa cervical cancer cells using an MTT assay. The cells were incubated with purple nutsedge essential oil in a 96-well plate with eight series of doses (3.9-500 μg/ml), and 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 (IC_{50}) using probit regression analysis. This research conclude that purple nutsedge essential oil IC_{50} on HeLa cells is 35.062 + 11.258μg/ml. This indicates that there is a cytotoxic effect of purple nutsedge essential oil on the HeLa cervical cancer cell line.

Keywords: *Cyperus rotundus* L., purple nutsedge, HeLa, cervical cancer

INTRODUCTION

Cancer is still a major problem in the health sector due to its increasing prevalence rate. Death rates due to cancer are also still very high. In 2012, about 8.2 million deaths in the world were caused by cancer. The prevalence rate of cancer in the population of Indonesia in 2013 amounted to 1.4‰, or 347,792 people. From the 2013 data, cervical and breast cancer prevalence rates are the highest among all types of cancer in women in Indonesia. Women with cervical cancer amounted to about 0.8‰ of women population and those with breast cancer totaled 0.5‰ (Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia, 2015).

Cancer treatment is currently carried out in a number of ways, including surgery, radiotherapy, chemotherapy, hormonal therapy, biological therapy and precision medicine (Hilli et al., 2010; Ramu and Jayanthi, 2017). Some of the chemotherapy 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 toxicity (Hosseini, 2015). Other cancer treatment problems include the high cost of treatment, relapse among patients who had improved, and a decreased quality of life (Kundu et al., 2014; Gautam et al., 2014). These problems have prompted researchers to explore natural materials to discover new anticancer substances with higher efficacy and more minimal side effects. Many natural materials are good sources for the development of medicines for various diseases (Hosseini, 2015; Gautam et al., 2014).

Many plants have been studied both *in vitro* and *in vivo* and many of them have potent chemopreventive and chemotherapy (anticancer) effects by reducing proliferation, inducing apoptosis, slowing metastasis, and inhibiting angiogenesis (Galati and O'Brien, 2004; Hosseini, 2015). Some plants are known to have a fairly selective cytotoxic effect by inducing apoptosis in cancer cells but not in normal cells. This has encouraged the continuous screening of anticancer agents that may induce apoptosis and are derived from plants, either as extracts or active compounds isolated from them (Taraphdar et al., 2001).

One of the medicinal plants that has the potential to be developed as an anticancer substance is purple nutsedge (*Cyperus rotundus* L.). 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 *purple nutsedge*. This plant's is

potential to develop because it is cheap and easy to obtain. Purple nutsedge is a wild grass that is scattered among various places in the tropics and grows in the lowlands up to a height of 1000 m above sea level. This grass 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 gardens (Sudarsono et al., 1996). 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 sedative (Susianti 2009; Sivapalan, 2013; Singh et al., 2012).

Purple nutsedge is classified in the Spermatophyta division, Angiospermae subdivision, Monocotyledonae class, Cyperales order, Cyperaceae family, Cyperus genus, and *C. rotundus* L. species. The purple nutsedge plant grows to a height of about 40 cm. Its trunk is soft, triangular, and pale green. The leaves are green, single, and oblong shaped, with a tapered tip and flat edge. Their average length is ±50 cm and width is ±5 mm. The purple nutsedge flower is at the end of the stem, brown, and grain shaped. The flower is 1-3 cm in length and ±2 mm in width, has three stamens, red anthers, and pistils that are ±1.5 cm long. The fruit is oval, 1.5 cm long ±, and brown. The roots are fibrous and white. The tuber 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 bitter (Anonymous, 2000; Sudarsono et al., 1996). A variety of studies have been done on the purple nutsedge tuber as an anticancer substance, but not on the effects of its essential oil on cervical cancer, which was investigated in this research.

MATERIALS AND METHODS

Plant Material: 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 were 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 were 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 $MgSO_4$ $7H_2O$ 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.

Cytotoxicity Test with MTT Assay: Based on Mosman (1983), 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 10^4 cells dissolved in 100µl culture medium (RPMI 1640) containing 0.5% FBS (fetal bovine serum). The cells were then incubated (starvation) for 24 hours in a 5% CO^2 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 (purple nutsedge tuber essential oil) was done in 8 serial doses 3.9; 7.8; 15.6; 31.2; 62.5; 125; 250; 500µg/ml and doxorubicin (positive control) in 8 serial doses 0.625; 1.25; 2.5; 5; 10; 20; 40; 80µg/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 10µl MTT solution were added to each well and then incubated for 4 hours in a 5% CO^2 incubator at 37°C. After that, 100µl 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:

$$\frac{(A-B)}{(C-B)} \times 100\%,$$

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 IC^{50}, which is the concentration of each test material that causes the number of living cells about 50%, was obtained.

RESULTS AND DISCUSSION

The cytotoxic activity of the purple nutsedge essential oil as shown by a dose-response curve can be seen in Table 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.

Table 1: HeLa Cell Viability After Treatment with Essential Oil

Essential oil concentration (µg/ml)	Average of cell viability (%) sel (%)	Standard deviation
1.81	81.681	1.972
3.9	95.623	14.335
15.63	96.650	13.006
31.5	86.976	18.240
62.5	44.664	22.025
125	-0.784	0.337
250	-0.297	1.228
500	1.324	0.521

The viability of Hela cell after treatment with 1.81µg/ml of the purple nutsedge essential oil is 81.681%, whereas the viability after treatment with 500µg/ml of the purple nutsedge essential oil is 1.324%. This shows that the essential oils have cytotoxic activity in the HeLa cells. From the percentage of cell viability, the inhibitory concentration (IC^{50}) can be calculated using probit regression analysis, and the obtained IC^{50} of essential oil to HeLa cells is 35.062 ± 11.258µg/ml. The cytotoxic activity of doxorubicin (positive control) is shown in Table 2.

Table 2: HeLa Cell Viability After Treatment with Doxorubicin

Doxorubicin concentration (µg/ml)	Average of cell viability (%)	Standard deviation
0.625	68.76	5.85
1.25	70.19	1.18
2.5	57.58	2.77
5	53.03	2.59
10	50.28	0.82
20	43.38	0.98
40	28.37	1.92
80	8.80	0.53

The viability of Hela cell after treatment with 0.625µg/ml of the doxorubicin is 68.76%, whereas the viability after treatment with 80µg/ml of the doxorubicin is 8.80%. The percentage of cell viability can be calculated. The inhibitory concentration (IC^{50}) can be calculated using probit regression analysis, and the IC^{50} of doxorubicin to HeLa cells is 5.588 ± 0.490µg/ml. The comparative curve between the 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.

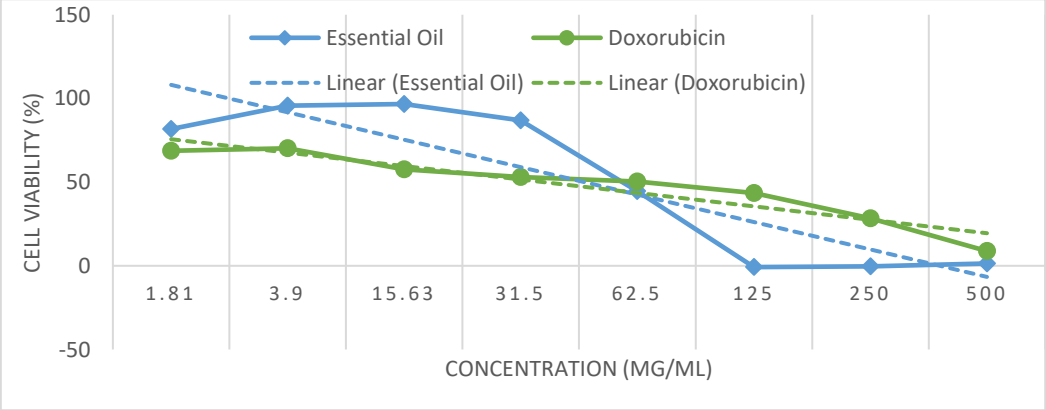

Figure 1: Dose-Response Curve of Essential Oil and Doxorubicin on HeLa Cells

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 (IC^{50}) < 50µg/ml (Mans et al., 2000). If a compound has IC^{50} > 100µg/ml, then it has a

weak cytotoxic effect, whereas if it has IC^{50} > 400μg/ml, it is not toxic (Mathabe et al., 2008). This means that the essential oils tested in this study have a strong cytotoxic effect and the potential to be developed as an anticancer substance. 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 IC^{50} (Kilani et al., 2008a).

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 L1210 (Soumaya et al., 2014). Sayed et al. (2007) proved that steroid glycosides from the purple nutsedge stem have a cytotoxic effect on mouse lymphoma cells (L5178Y). Kilani et al. (2008a, 2008b) 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 extract (Susianti, 2009).

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 oils (Singh et al., 2012; Zhou and Yin, 2012; Soumaya et al., 2014). 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 decreesing Bcl-2 protein (Gautam et al., 2014). 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, patchoulinenone, aristrol-9-en-3-one, selina-4, 11 diene, aristrol-9-en-8-one, kobusone, sugetriol, isokobusone, isocyperol, sugeonol, and sitosterol (Singh et al., 2012). 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-Cyperone (Lawal and Oyedeji, 2009) 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 world (Al-Massarani, 2016). 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

This research found that purple nutsedge essential oil IC_{50} on HeLa cells is 35.062 + 11.258 μg/ml), which means that purple nutsedge essential oil has a cytotoxic effect on the HeLa cervical cancer cell line.

REFERENCES

Al-Jumaily, E.F.A., J.K.T. Al-Isawi, Chemical composition and antioxidant potential of polyphenol compounds of *Cyperus rotundus* L. Rhizomes. AJPCT 2(11): 1277–1286 (2014).

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).

Anonymous, *Inventaris Tanaman Obat Indonesia (I).* Vol 1. Departemen Kesehatan dan Kese-

jahteraan Sosial RI, Badan Penelitian dan Pengembangan Kesehatan, Jakarta (2000).

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).

Gautam, N., A.K. Mantha, S. Mittal, Essential oils and their constituents as anticancer agents: A mechanistic view. *BioMed Research International* 1–23 (2014).

Hilli, Z.A, E.F. AL-Jumaily, N.Y. Yaseen, Role of volatile oils fraction of *cyperus rotundus* in induction of apoptosis on cancer cell lines *in vitro. Iraqi J. Biotech.* 9(2): 286–298 (2010).

Hosseini, A.G., Cancer therapy with phytochemicals: Evidence from clinical studies. AJP 5(2): 84–97 (2015).

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).

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).

Kundu, J., K. Chuna, O.I. Aruomab, J.K. Kundua, Mechanistic perspectives on cancer chemoprevention/chemotherapeutic effects of thymoquinone. *Mutation Research* 768: 22–34 (2014).

Mans, D.R.A., A.B. Da Rocha, G. Schwartsmann, Anti-cancer drug discovery and development in Brazil: Targeted plant collection as a rational strategy to acquire candidate anti-cancer compounds. *Oncologist.* 5: 185–198 (2000).

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).

Mosman, T., Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65: 55–63 (1983).

Pusat Data dan Informasi Kementerian Kesehatan RI, *Stop Kanker* http://www.depkes.go.id/resources/download/pusdatin/infodatin/infodatin-kanker.pdf. Diakses tanggal 4 November (2015)

Ramu, G., A. Jayanthi, A review on precision medicine and its advantages. *Pak. J. Biotechnol.* 14 (3): 515-519 (2017)

Sayed, H.M., M.H. Mohamed, S.F. Farag, G.A. Mohamed, P. Proksch, A new steroid glycolside and furochromones from *Cyperus rotundus* L. *Nat Prod Res* 21(4): 343–350 (2007).

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).

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).

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).

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).

Susianti, Selektivitas ekstrak umbi rumput teki (*Cyperus rotundus* L.) terhadap sel HeLa dan SiHa serta pengaruhnya terhadap apoptosis. *Thesis,* Universitas Gadjah Mada, Yogyakarta (2009).

Taraphdar, A.K., M. Roy, R.K. Bhattacharya, Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. *Curr Sci* 80(11): 1387–1396 (2001).

Zhou, Z., W. Yin, Two novel phenolic compounds from the rhizomes of *Cyperus rotundus*. *Molecules* 17(11): 12636–12641 (2012).