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

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INTRODUCTION

Cancer is still a major problem in the health sectorduetoitsincreasingprevalencerate.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, cervicalandbreastcancerprevalenceratesarethe highest among all types of cancer in women in Indonesia. Women with cervical cancer amountedtoabout0.8‰ofwomenpopulation,andthose with breast cancer totaled 0.5‰ (Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia,2015).

Cancer treatment is currently carried out in a numberofways,includingsurgery,radiotherapy, chemotherapy, hormonal therapy, biological therapy and precision medicine (Hilli et al., 2010; Ramu and Jayanthi, 2017). Some of the chemotherapy drugs often used includeantimetabolites, 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 thehighcostoftreatment,relapseamongpatients whohadimproved,andadecreasedqualityoflife (Kundu et al., 2014; Gautam et al., 2014). These problems have prompted researchers to explore naturalmaterialstodiscovernewanticancersubstances 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*invivo*andmanyofthemhavepotentchemopreventiveandchemotherapy(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 cellsbutnotinnormalcells.Thishasencouraged thecontinuousscreeningofanticanceragentsthat 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*.InEnglishitisoftencalled*nutgrass*,*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'sis

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 growsonunusuallydryfarmland,infields,andin gardens(Sudarsonoetal.,1996).Ithaslongbeen used as a remedy for various diseases, such as diarrhea, inflammation, diabetes, fungus, and cancer; has antimicrobial, antioxidant, antimutagenic,antipyretic,analgesic,anti-emetic,andanti -obesity effects; and can be used as a stimulant, diuretic, and sedative (Susianti 2009; Sivapalan, 2013; Singh et al., 2012).

PurplenutsedgeisclassifiedintheSpermatophytadivision, Angiospermaesubdivision, 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 oblongshaped,withataperedtipandflatedge.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 cminlengthand±2mminwidth,hasthreestamens,redanthers,andpistilsthatare±1.5cmlong. 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 andthe outsideisbrownwhiletheinsideiswhite,similar to spices; it tastes bitter (Anonymous, 2000; Sudarsono et al., 1996). A variety of studieshave 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 thisresearch.

MATERIALS AND METHODS

PlantMaterial: Thepurplenutsedgetubersused in this study came from the wild areas surrounding Bandar Lampung City, Lampung Province, Indonesia.Theinitialprocessinthisresearchwas to identify and determine which plants would be used based on the observation of plant physiologicalcharacteristicssuchas flowers, leaves, stems, roots, and tubers. The next step was to ensurethe 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 thisdetermination 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, afterwhichtheywerecutintosmallsizes.Atotal of10kgofdrytuberswerедistilledwithaqua2/3 of pumpkin contents for approximately 4 hours. Furthermore, the essential oil, which was still mixedwithalittlewater,wasremovedbyadding $MgSO_4$ $7H_2O$ 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 glassbottles.

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.Itwasperformedusinga96-wellmicroculture plate.Eachwellintheplatewasfilledwitha HeLa cell suspension of 2 x10^4 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 mediaineachwellwasremoved,thenreplacedwith new media containing 10% FBS and treatment withmaterialtest(purplenutsedgetuberessential oil)wasdonein8serialdoses3.9;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 addedtoeachwellandthenincubatedfor4hours in a 5% CO^2 incubator at 37°C. After that, 100µl ofsodiumdodecylsulfate(SDS)wasaddedamounted 10% in 0.01% HCl and then themicroplate 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) wasobtained by theformula:

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

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 3as 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 concentrationofeachtestmaterialthatcausesthe number of living cells about 50%, wasobtained.

RESULTS AND DISCUSSION

The cytotoxic activity of the purplenutsedge 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 with500µg/mlofthepurplenutsedgeessentialoil is1.324%.Thisshowsthattheessentialoilshave 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 Table2.

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/mlofthedoxorubicinis68.76%,where- as 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 essentialoilsanddoxorubicinisillustratedinFigure1. The curve shows that increasing the concentrationofthepurplenutsedgedosescandecreasethe viability of HeLacells.

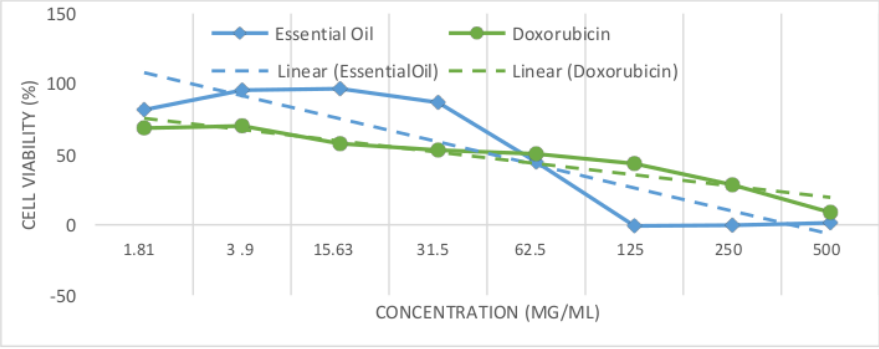

Figure 1: Dose-Response Curve of Essential Oil and Doxorubicin on HeLa Cells

Based on the standards of the National Cancer Institute(NCI)intheUnitedStates,anextracthas quitealotofpotentialtobedevelopedasan 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 researchwherepurplenutsedgetuberessentialoil was investigated and found to have a very strong cytotoxic effect on murine lymphoblastic leukemia(L1210)cells.However,thatresearchdidnot provide information about IC^{50} (Kilani et al., 2008a).

In some studies, purple nutsedge was shown as having cytotoxic effects on cancer cells, thus revealingitspotentialfordevelopmentasananticancer 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 apoptosisin L1210(Soumayaetal.,2014).Sayedetal.(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 theextract hasacytotoxiceffectbyinducingapoptosis.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 havecytotoxiceffectsonHeLaandSiHacervical 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 inflammationthatcancausecancer.Avarietyofstudies have identified various compounds in purplenutsedge in the form of antioxidants and variuous compounds that are suspected to have medical effectsandthepotentialtobedevelopedasdrugs. 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 havebeenwidelystudiedtohaveanticancereffe- cts as both antioxidants and triggers ofapoptosis. 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 isolatedfrompurplenutsedgeareα-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 (Singhetal.,2012).Differencesinthesoilconditions, 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 partsof Africa,thesameprimarycompoundsofcyperene 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 percentageoftheircompoundswhencomparedwith other C. *rotundus* essential oils from different areas around the world (Al-Massaran 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 essentialoilIC$_{50}$onHeLacellsis35.062+11.258 µg/ml), which means that purple nutsedge essentialoilhasacytotoxiceffectontheHeLacervical cancer cell line.

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