

Influenced of Epiphyte Flavonoid Tea *Scurrula Artropurpurea* Danser in Apoptosis Induction for Tissue Isolation of Cervical Cell Cancer

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Abstract: *Cervical carcinoma is a type of cancer to two experienced by women and the leading cause of death for women in the world, extracts of loranthus tea (*Scurrula artropurpurea*) have flavonoids, compounds to which this activity in flavonoids induces apoptosis and suppresses the proliferation in cervical carcinoma cell culture. This research aims to identify the potential of the flavonoid compounds in tea induce apoptosis loranthus and inhibit proliferation in cervical carcinoma cell culture. This type of research is used in experiments which is conducted in the laboratory of Faculty of Pharmacy University of Airlangga. The sample used is cervical carcinoma HeLa cells (the cells He La). These cells are epithelial cells morphology that already infect Human Papilloma Virus (HPV) type 18. Then do a test with a Sitotoksitas Assay and MTT Assay Apoptosis by Double Staining Method. The results of the research on proliferation and apoptosis in cells was reported in descriptive as follows: 1) Extract tea *Scurrula loranthus artropurpurea* (Blume) Danser good fraction of ethanol, chloroform, or n-heksan was able to inhibit proliferation with the strongest potential shown by extract chloroform with IC50 values of 96.160 μ g/ml of ethanol, followed by the value of the IC50 of μ g/ml 298.801 and n-heksan with IC50 values of 489.661 μ g/ml. 2) Extracts tea *Scurrula loranthus artropurpurea* (Blume) Danser good fraction of ethanol, chloroform, or n-heksan is able to induce apoptosis in the cell He-La has the potential for chloroform Fraction. Induces apoptosis levels where most powerful 22.413 μ g/ml (23.31% of IC50) able to cause apoptosis of 75.72%, followed by the tea ethanol fraction loranthus and n heksan. Further research is needed to find out the mechanism of flavonoid in inhibiting the proliferation and inducing apoptosis in cervical cancer cells.*

Keywords: Loranthus extract tea (*Scurrula artropurpurea*), Flavonoids, Apoptosis, Proliferation, Cell cervical carcinoma

1. Introduction

Cervical carcinoma is a type of cancer to two experienced by women and the leading cause of death for women in the world. In the area of cervical cancer incidence in Southeast Asia tops the list i.e 180,000 incident of the total incidence of cancer, namely a number of 1,726,000 the incident (WHO, 2008, Gonzales et al, 2005). In Indonesia the cancer is the fourth leading cause of death after a stroke, hypertension and diabetes mellitus. The cause of death of those deaths caused by cervical cancer, breast cancer and lung cancer was 7.7% (Badan Penelitian Pengembangan Kesehatan, 2008).

Cervical carcinoma is primary cancer of the cervix (the cervical canal and or porsio). Infection of Human Papilloma Virus (HPV) are found at 99.7% of cervical HPV infection so carcinoma is the main risk factor in the development of cervical cancer is mainly a type 16 and 18 which includes the types of high risk HPV. Another risk factor is the age when you first had sexual intercourse, number of partners, multiparitas, hormonal contraceptives, sexual intercourse and smoking (Joe and Jae, 2005, Gillison, 2008, Alba et al., 2009, Andrijono, 2010).

Loranthus has been used traditionally to overcome some of the disease include diarrhea, worms, cough, diseases of tonsils and smallpox. But studies on loranthus is also evolving towards utilization of loranthus as a remedy for cancer diseases either in vitro or in vivo. Anticancer activity of loranthus is related to the content of the active compounds contained on loranthus.

Several active compounds have been identified on loranthus *Scurrula artropurpurea* that comes from the tea plantation in Lembang, Bandung was the flavonoids like quercetin, catechin, monoterpen glucosides, lignan glycosides, some groups of fatty acids, flavan compounds and (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-O-gallate, (-)-epigallocatechin-3-O-gallate (EGCG) (Mills et al., 2001, Ohashi, et al. 2003).

Flavonoid has been known to function as antibacterial, antiviral, bitter taste, antialergi, antineoplastik and Antidiarrhoeal activity, as an antioxidant (Mills et al., 2001). Test compounds a flavonol (Quercetin) from *Scurrula ferraginea* Danser on cancer cells showed quersetin cytotoxic effect on glioblastoma cells/U251 (Le Devehat et al, 2002). Loranthus extract tea (*Scurrula artropurpurea*) has the effect of prophylactic and curative towards karsinogenesis the nasopharynx in C3H mice (Sulistyo, 2008). The methanol extract of leaves of Duku loranthus (*Dendrophoe* sp) have a drag on growth in myeloma cell culture in vitro (Lazuardi, 2006). The content of fatty acids and flavonoids extract of leaves of *Scurrula artropurpurea* activity inhibits the growth of cancer cells in the rat mesotelial cell culture (Ohashi et al., 2003). Other research shows a water extract of tea *Scurrula loranthus oortiana* and *S. junghunii* can ruin a fibrosarcoma tumor cells directly and increase the sensitivity of the tumor cells against TNF α molecules (Yudiarti and Muwarni, 2002).

One species loranthus in Wonosari is *Loranthus Scurrula* Tea *artropurpurea* (Blume) Danser. In previous research has successfully identified flavonoids compounds on loranthus is with thin layer chromatography examination and

spectrophotometer is a flavonol, flavones, catechins, dihidroflavonol and flavanon. The research that will be done is to test the potency of flavonoids on loranthus is particularly through its activity in inducing apoptosis and suppresses the proliferation in cervical carcinoma cell culture.

2. Method of Research

This research aims to know the potential of the flavonoid compounds in tea induce apoptosis loranthus and inhibit proliferation in cervical carcinoma cell culture. This type of research is used in laboratory experiments.

In this study used cells of cervical carcinoma HeLa (cell He La). These cells are epithelial cells morphology that already infect Human Papilloma Virus (HPV) type 18. Variable in this research are apoptosis and proliferation in cervical carcinoma cancer cells after the granting of loranthus tea flavonoids. In this study the tools used are: Mikropipet 10 μ l, 100 μ l, 1000 μ l, Cyro tube, shelves of small, 96 cryo wll plate, Conical tube, a Yellow tip and blue tip, ELISA reader. The materials used are: Phospat buffer saline 1 x, complete culture Media (MK), DMSO, MTT 0.5 mg/ml PBS, 10% SDS in 0.1 N HCl, Tissue, and aluminum foil.

The research was conducted using a cell culture derived from a CO2 incubator, with the condition of 80% konfluen for harvested. Calculated cell number and serial dilution is made on the COURT as required following the Protocol of counting cells. Cells transferred into sumuran, each 100 μ l. each charging 12 sumuran, resuspensi agar cells remains homogeneous. Will stay at 3 pieces sumuran blank with no cells, as a media control. Carried out observations of the State of the cells on the microscope to view the cell distribution is inverted and then documented. Cells incubated in incubator overnight, in order for recovery after a harvest. Before the treatment, the conditions observed cells come back and certainly have recovery his condition, which has been under konfluen 80%.

If the conditions are still not allowed to be used, incubation done again. After the cells are in the expected conditions, created a series of sample concentration for treatment, including for the control of cells and the control of DMSO, in accordance with the sample preparation protocol. Plate contains a cell taken from a CO2 incubator, then put the sample into a concentration series sumuran (triplo). Incubated into CO2, overnight. Towards the end of incubation time, the documentation of the condition of the cell for each treatment. Media cell disposed of, by means of reverse plate with angle of 180o above exile with a distance of 15 cm, then the plate is pressed gently on top of tissue packed to drop sweet residual liquids.

On *sumuran* a filled cell, put 100 μ l of PBS then disposed of by way of a reverse plate with angle of 180o above exile with a distance of 15 cm, then the plate is pressed gently on top of tissue packed to drop sweet residual liquids. This procedure is repeated once more. Prepared reagent MTT 0, 5 mg/ml in PBS for the treatment, by the way is taken from the stock solution 10 ml MTT plus 10 ml MK (for 1 piece

plate contents 96 well). Gloves used in the procedure mandatory addition of MTT, as MTT are carcinogens. MTT reagent is added as much as 110 μ l into every sumuran, including control of the media (without cells). Cells incubated during 2-4, in 5% CO2 incubators, Incubation done to formazan formed. The formation of the formazan is observed by using the inverted microscope.

If you've added the solution stopper formazan formed 100 μ l of 10% SDS in 0.1 N HCL. This procedure does not work for perly carried out in LAF. Plate wrapped in paper or aluminum foil, and then incubated in a dark place, with room temperature during the night. After incubation is complete, the wrapper and cover plate removed, and inserted into the ELISA reader. Done reading absorbance for each sumuran at $\lambda = 550-600$ nm (595 nm). The resulting data is used, the calculated percentage of live cells and analyzed with the SPSS IC50 price probit.

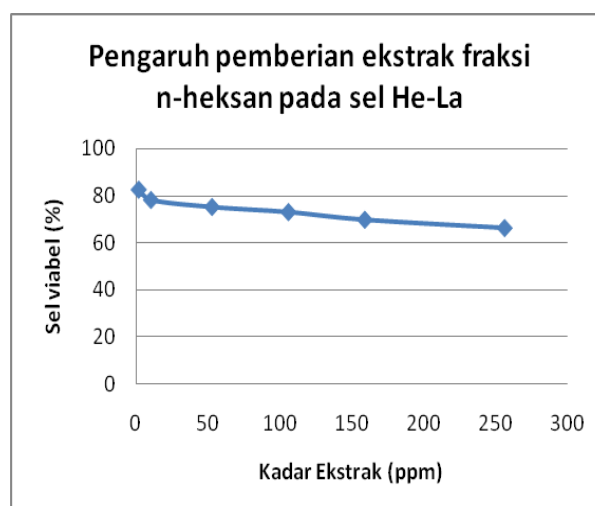
3. Result

The potential to inhibit the proliferation of Loranthus tea.

a. MTT test assay Results heksan n fraction sitotoksitas extract loranthus tea with the fraction of n-Heksan against the cell He-la can be seen in table 4.1:

Nu	Levels (ppm)	Absorbance (595 nm)	Alive cell (%)
1	265,00	0,278	66,22
2	159,00	0,291	69,73
3	106,00	0,303	72,97
4	53,00	0,311	75,13
5	10,60	0,322	78,10
6	2,12	0,338	82,43

In order to know the value of IC 50, the data above will be analyzed by using probit software SPSS.



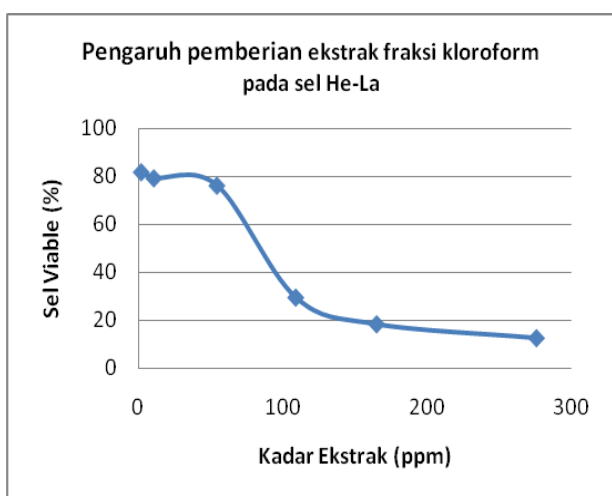
From the picture 4.2 Note that the higher the levels of extract, then viability cell He-la on the wane. Based on the calculations, it is known that the values of IC50 for extract fraction n-heksan is 489.66 ppm

b. MTT Test of fraksi kloroform

Result of sitotoksik test of Tea Loranthuswith kloroform fraction to the cell He-la can be seen on the table 4.2 below:

	Level (ppm)	Absorbance(595 nm)	Viable cell (%)
1	272,50	0,079	12,70
2	163,50	0,097	18,47
3	109,00	0,129	29,64
4	54,50	0,316	76,31
5	10,90	0,341	79,37
6	2,18	0,391	81,89

To find out the values of IC50 data above will next analyzed using the SPSS Software probit regression. Calculation based on the value of IC50 for the fraction of chloroform is 96.150 μ g/ml. Graph influence awarding of chloroform fraction loranthus tea can be seen in Figure 4.3.

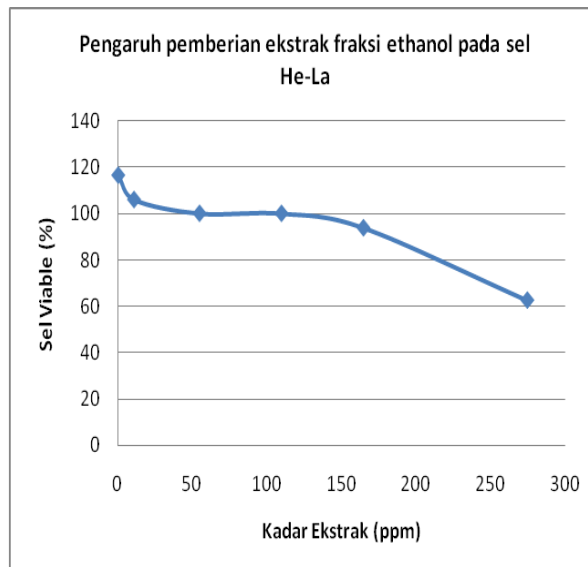


c. MTT Test fraction etanol

Test results sitotoksitas extract loranthus tea with ethanol fraction against the cell He-la can be seen in table 4.3:

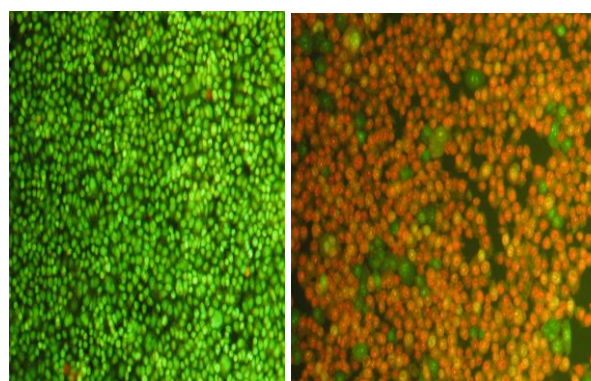
No	Level (ppm)	Absorbance (595 nm)	Viable cell (%)
1	275,00	0,256	62,52
2	165,00	0,366	93,78
3	110,00	0,383	100,00
4	55,00	0,415	100,00
5	11,00	0,428	105,95
6	0,44	0,464	116,47

Probit analysis and based on the use of SPSS software, obtained that the use of the extract fraction of ethanol will reach 298.801 levels on the IC50 value of μ g/ml. ethanol fraction influence Graph granting loranthus tea can be seen in Figure 4.4 the following.



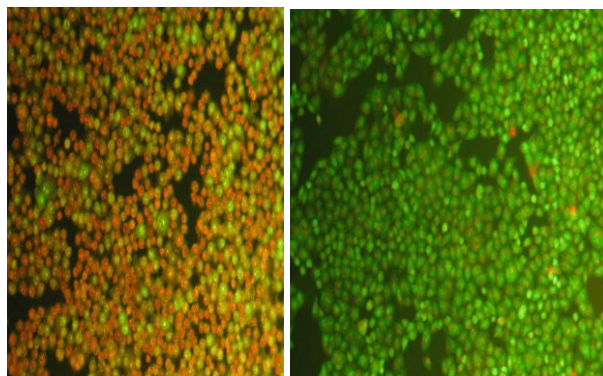
Of the three factions can note that the fraction of chloroform extract was the most powerful potential in inhibiting the proliferation of Hela cells marked with an IC50 value of lowest, namely 96.150 μ g/ml. Based on testing of TLC and ppektrofotometer in chloroform fraction has a content of flavonoids: flavonon, dihidroflavonon, and catechins. The potential of the adahal fraction of ethanol and next is the fraction of n-heksan.

Research is also done in the test against the potential of loranthus extracts in inducing apoptosis by Double Staining method on the cell He-La observed Parameters. is He-La cell percentage who suffered death through apoptosis mechanism. In Figure 4.5 the following display measurement results on HeLa cells apoptosis after being given treatment tea loranthus fraction n-heksan, chloroform and ethanol. Fluorescence microscopy observations performed with at the time of 48 hours after the HeLa cell culture ditreatment by loranthus tea.



(A). Control

(D). Fraksi Etanol



(C). Fraksi Klorofom (B) Fraction n-Heksan

In Figure 4.5 can be seen that in accordance with the results of a test of sitotoksitas, that the granting of extracts tea loranthus is capable of inducing cell death. Visually, when compared with the control (A), then in the cell culture (B), (C) and (D) given that there is a growing loranthus extract quite significant on populations of red-coloured cells. Red cells indicate apoptosis, meaning giving of extracts tea loranthus is able to cause apoptosis in the cell He-La. in observation under the microscope, the cells glowed with the colour orange is a cell that allegedly undergoing apoptosis. On the image of A shown control data (condition shortly after harvest), it appears visually the population of green-coloured cells (viabel/live) dominate the entire population of cells.

The calculation of the number of cells demonstrated that the alleged cell death due to apoptosis just 1.01%. In figure (B) with the granting of extracts of n-heksan with 225 ppm levels, green cell population decreased significantly, with a predominance of green-coloured cells that start memendarkan orange color, marker started to undergo apoptosis. The population of cells undergoing apoptosis is known as much as 14.92%. In figure (C) shows the State of the cell populations after the given extract with chloroform levels 22.413 ppm, green cell population decreased significantly, with a predominance of red cells. The population of cells undergoing apoptosis is known as much as 75.72%. While in figure (D) by administering ethanol extract with 231 ppm can be seen that the population of the green cells decreased significantly, with a predominance of cells of the colour orange. The population of cells undergoing apoptosis is known as much as 85.44%. Based on the results of the examination of proliterasi and apoptosis then 4.4 relationship between the IC50 values shown loranthus tea with the ability to induce apoptosis.

Fraction	Compound	IC ₅₀ (µg/ml)	Apoptosis	
			Epiphyte level (µg/ml) (from IC ₅₀)	% apoptosis
N heksan	<ul style="list-style-type: none"> • Flavanon • Dihidroflavonol • Flavon 	489,66	225 (45% from IC ₅₀)	14,92
Kloroform	<ul style="list-style-type: none"> • Flavanon • Dihidroflavonol • Katekin 	96,16	22,413 (23,31% from IC ₅₀)	75,72
Etanol	<ul style="list-style-type: none"> • Flavonol • Flavon • EGCG 	298,81	213 (71,85% from IC ₅₀)	85,44

From the above data can be deduced loranthus faction chloroform has potential induces apoptosis levels where most powerful 22.413 µ g/ml (23.31% of IC50) able to cause apoptosis of 75.72%, followed by the tea ethanol fraction loranthus and n heksan.

4. Discussion

From the results of research that has been done, it is known that the extract of loranthus is able to exert influence on cytotoxic cell He-La. This is because the content of the flavonoids in tea loranthus allegedly can play a role in inhibiting cancer cell proliferation in nature. Barriers against the proliferation of cancer cells is thought to relate to the mechanisms of induction of apoptosis.

Some preliminary research has been done, it has been concluded that flavonoids have the potential of anti proliferation and induction of apoptosis (Hodek et al, 2002, Kanadasami et al, 2003, Ren et al 2003, Androutopoulos et al, 2010, Chahar et al, 2011). So in accordance with the previous data and based on the value of the IC50 of the three extracts used in this study, it can be stated that tea flavonoids content of loranthus is able to bring up the effect of cytotoxic in a dose dependent manner.

Chloroform extracts known to have the most powerful cytotoxic effects, with IC50 values of 96.160 µ g/ml of ethanol, followed by the value of the IC50 of µ g/ml 298.801 and n-heksan with IC50 values of 489.661 µ g/ml. The efficiency of administering the lowest doses but was able to bring up the cytotoxic influence is the basis to argue that researchers on research this time, extract the most klorofom faction loranthus potentially cytotoxic agents on cell He-La. But still need to test more in depth about the influence of each extract against cell healthy that there are cancer cells in vivo conditions.

From the results of the validation of cytotoxic capability via the apoptosis, the alleged ability of cytotoxic fraction extract third loranthus tea can affect the induction of cell death via apoptosis in a dose dependent manner. When seen on the results of the photo then the number of cells that viabel will decrease significantly after the giving of extracts tea culture on loranthus, although molecular mechanism of the apoptosis due to induction by flavonoids has yet to be explained clearly. Transcription factor kappaB (NF-kappaB), endonuclease activation, dan protein supretion Mcl-1 protein. Some of the mechanisms that have been known to include the existence of barriers against DNA topoisomerase enzymes work I/II, a decrease in the production of reactive oxygen species (ROS), the regulation of the expression of heat shock proteins, regulation of signaling pathways, activation of caspase-9 and caspase-3 in stages which result in the release of cytochrome C, down regulation of the expression of Bcl-2 and Bcl-X (L) and the promotion of the expression of Bax and Bak, activation nuclear transcription factor kappaB (NF-kappaB) endonuclease activation, suppression, and Mcl-1 protein-protein

In this research it can be dinnyatakan that the third extract loranthus tea good fraction of ethanol, n-heksan, or chloroform, have the potential to induce apoptosis in a dose dependent manner, but more research is still needed in order to know to extract the most potential, and the initial mechanism.

HeLa cells are known to express 2 oncogenes E6 and E7, IE. E6 bind to p53 that terfosforilasi while the E7 protein bound to pRb, where pRb and p53 is a tumor suppressor gene that is associated with the development of cervical carcinoma.

On a normal p53 will experience upregulasi after damage DNA because DNA damage will induce the activation of p53 that would result in the termination of the cell cycle at the G1 stage or apoptosis. Termination of this cycle allows the cell to perform DNA repair and DNA repair cannot be done then it will do the process of apoptosis. The HPV E6 protein will bind to the E6-AP and p53 p53 will experience so ubiquitinasi so it will fit in a series of stages of degradation by proteolytic enzymes.

The role of protein E7 protein related to retinoblastoma (pRb). Phosphorylation on pRB sequentially by cyclin/cdk complexes, will produce the resistance activities represor from pRb. E7 protein HPV will bind to the protein pRb terfosforilasi, where this would disrupt complex bonds pRb with cellular E2F transcription factors so that the E2F transcription factors will escape from the complex and the cell will enter into phases-phase synthesis (S phase) in the cell cycle and the pRb will be degraded.

Thus if there are obstacles on the swb p53 and pRb by HPV will can lead to uncontrolled cell cycle, DNA repair is not happening and not happening on the development of Impaired apoptosis cell cycle can occur due to the effects of antikarsinogenik flavonoids. Stages of DNA synthesis (S phase) and the separation of the two child cells (M phase) is two processes are important in the developmental process of the cells. The time between phases of the S and G2 phases known as M where the phase is important because it determines the ability of cells to repair errors during duplication of DNA and prevent the occurrence of such error to decrease cell child is. While the G1 phase is the phase where the main proceedings occurred will occur from the stages of development in the cell cycle that will separate the phases of the M and S phases as preparatory phase cells will do DNA duplication through signal mitogen.

CDK (Cyclin dependent Kinase) is known to be a key regulator in the developmental process of the cell cycle. The existence of changes in activities of CDK is a marker of the presence of neoplasia process. Various types of cancer known to be associated with excessive activity of CDKs, where this incident is caused by mutation in the gene or gene inhibitors of CDKs CDKs. Therefore, compounds that inhibit or change the nature of the activity of CDK needs to be explored more deeply to find new cancer therapy agents. Checkpoints in G1/S and G2/M cell cycle in cancer cells in culture are known to be affected by flavonoids among other types of silymarin, genistein, quercetin, kaempferol, luteolin, daidzein, apigenin, and epigallocatechin 3-gallate. Various studies of

laboratory found that flavopiridol can induce cell cycle arrest in the G1 or G2/M due to the inhibition of CDKs.

Possibility of flavonoids compounds on ftaksi n heksan, chloroform and ethanol also has potential as an inhibitor of the activity of the CDKs that can inhibit the proliferation and enhance apoptosis. Of research results obtained fraction has the strongest potential of chloroform in inhibiting the proliferation and inducing apoptosis in HeLa cells when compared with the fraction of n heksan and the fraction of ethanol. It is closely related to the content of flavonoid compounds from the faction. In the fraction of n heksan contains flavanon, dihidroflavonol and flavones, whereas chloroform fraction contained compounds flavanon, dihidroflavonol and catechins. The fraction of ethanol contains EGCG, a flavonol and flavones. Based on this data then it is likely a strong potential on the ffraksi chloroform caused due to the presence of catechins.

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