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The potential of understorey plants from Gunung Gede Pangrango National Park (West Java, Indonesia) as cervixs anticancer agents

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Abstract. *Arbiastutie Y, Marsono Dj, Hartati MS, Purwanto R. 2017. The potential of understorey plants from Gunung Gede Pangrango National Park* (*West Java, Indonesia*) *as cervixs anticancer agents. Biodiversitas 18*: *109-115.* This study aims to screening 97 species obtained from the understorey plants of The Gunung Gede Pangrango National Park of West Java, Indonesia as the anticancer cervixs agents. The anticancer plant activity is determined by the cytotoxicity assay of understorey plant methanol extracts against HeLa cervixs cancer cells using MTT test method. IC_{50} which is an indicator of cytotoxicity is determined by the probit analysis. The results show that of the 97 plants tested, there are five potential anticancer plants i.e. *Physalis peruviana* L. (Solanaceae), *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), *Lantana camara* L. (Verbenaceae), *Clidema hirta* (L). D.Don (Lamiaceae), *and Solanum torvum* Sw (Solananceae) with IC_{50} values of 67.85 µg/mL, 3.38 µg/mL, 43.54 µg/mL, 36.93 µg/mL, and 59.09 µg/mL respectively. The phytochemical compound find five understorey plants consisting of alkaloids, steroids, terpenoids, phenol, flavonoids and tannin.

Keywords: Anticancer, Gunung Gede Pangrango National Park, HeLa, MTT assay, understorey plant

INTRODUCTION

Tropical rain forest ecosystem and the entire biodiversity in it have the important value for human life and the environment, among others, as a source of natural resource for human life, the site of the hydrological cycle, the cycle nutrients, protecting it from flooding, erosion, drought and as a protector of the global climate change (Zaenuddin 2008). Based on the results of the inventory of potential diversity of medicinal plant species in various forest conservations of national parks in Indonesia, it shows that each unit of the national park finds various species of medicinal plants which can treat the illness of 25 groups of society (Zuhud 2008). Biodiversity both on trees, seedlings and understorey plants is an asset for the utilization of natural resources for various purposes (Matsjeh 2004).

Soerianegara and Indrawan (2008) state that the understorey as one of the members of the forest community, has an important role in forest ecosystems. The understorey plants besides having the ecological function, it has also be used as food, medicinal plants, and an alternative energy source (Hilwan et al. 2013). The understorey in the tropical rain forest stratification is placing in stratum D, which is a layer of shrubs, bushes and ground covering vegetation layer on stratum E (Soerianegara and Indrawan 2008).

Lower plants contain a variety of active compounds in the form of secondary metabolites such as alkaloids, flavonoids, steroids, triterpenoids, and coumarin (Dewick 1997; Cutler 2000; and Duke and Steven 1990). The content of these compounds have physiological effects and useful bioactive ingredients to be used as medicine, especially for cancer (Matsjeh 2004). Research that has been done previously shown that some plant species of understorey plants has a potential cytotoxic (Arbiastutie 2010). Utilization of understorey plants in tropical forest in Indonesia is still very limited and has not been developed. Some understorey plants in the forest ecosystem have a function as a cure for certain types of diseases, one of which is for treating cancer.

Currently, cancer is still a threat to human life. Death from cancer is expected to continue to increase every year. Various attempts have been made to the treatment of cancer including the chemotherapy. Cancer treatment with chemotherapy can harm the healthy body tissue and has no side effects occurrence of resistance to cancer drugs given (Katzung 1995). Methods such treatment still has weaknesses in terms of the high costs and side effects. Various obstacles and side effects are pushing the need for a new breakthrough cancer treatment method that has a high prospect and minimal side effects. One attempt to do to cope with the high cost of cancer treatment is to develop natural materials as anticancer agents.

One indicator of plant material as an anticancer agent is the nature of cytotoxicity. The potential cytotoxicity of understorey can be determined by testing the activity of bioactive compounds using cancer cells. Cancer cells are commonly used to look cytotoxicity properties of a material nature are HeLa cells. HeLa cell is a cell culture model of cervical cancer, which is generally used for the study because it is quite safe, grows faster and more easily handled (LabWork 2000). This research aimed to inventory the understorey plants from Gunung Gede Pangrango National Park and evaluation the cytotoxicity of understorey plants as anticancer cervixs against HeLa cell.

MATERIALS AND METHODS

Study area

The research sites in the National Park of Gunung Gede Pangrango (TNGGP) in West Java, Indonesia. TNGGP has an area of 22851.03 hectares. TNGGP topography varies from gently sloping to mountainous, with an altitude range between 700 m and 3000 m above sea level. The southern region, i.e. the area of Situgunung, has severe field conditions because there are hills that have a slope of about 20-80%. The type of soil that dominates TNGGP area is latosols brown, brown andosol associations and regosol brown, and gray and litosol regosol complex. The climate in the region TNGGP is based on the classification of Schmidt and Fergusson including the climate type A, with the values ranged between 11.30-33.30%, the air temperature ranged between 100-180°C, the average annual rainfall of 3000-4200 mm (Haris 2001). The field research activities are carried out in the zone of utilization of Cibodas Resort in Kampong Cibodas, Cimacan Village, Pacet Sub-district, Cianjur District, West Java of TNGGP with area 958.24 ha. The choice of location is based on the existence of the forest resources in the form of abundant understorey plants and it still has not been widely studied. The understorey plants are the inventory using the transect plot. All the leaves of understorey plant are identified expert at the Research Center for Biology, Indonesian Institute of Sciences, Cibinong Bogor, West Java, Indonesia.

Procedures

Preparation of understorey plants extracts

All the leaves of understorey plants samples are washed with the running water until clean and dried in an oven at 50-70°C. The dried leaf samples are milled using a grinder to be a powder. Five hundred grams of dried leaf powder are extracted by the maceration with 400 mL methanol for 3 x 24 hours. The samples are filtered and solvent extracts evaporated by using a rotary vacuum evaporator to obtain viscous extracts. Furthermore, the extracts are evaporated until it is dry and allowed to further used.

Cell culture

The HeLa cells are obtained from the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. HeLa cells are cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2% penicillin streptomycin, and 0.5% fungizone. Cells are incubated at 37°C and with a humidity of 5% CO_2 until they are confluent ± 80%.

Cytotoxic test with MTT Assay

Each methanol extract of the understorey plants as much as 10 µg is dissolved in 100 mL of dimethyl sulfoxide (DMSO) in order to obtain a test sample stock solution with a concentration of 100.000 µg/mL. After that the concentration test of each sample is made into 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL dam 31.25 µg/mL. A series of concentration of the understorey plants is then diluted in the culture medium, and it is having the incubation in CO_2 incubator. After 24 hour incubation, the medium is removed, and the cells are washed using PBS. Then 5 mg/mL of MTT on PBS is diluted by RPMI 1640, and 110 µl of reagent is added into each well. Then the micro plate is incubated in CO_2 incubator with 37^0C for 4 hours. After the incubation, the reaction is stopped by adding of SDS 10% in HCl 0.01 N. The microplate is then incubated overnight in room condition at dark place. Then the absorbance of each sample is measured using ELISA reader at λ 595 nm.

IC_{50} which is the cytotoxicity parameters are determined by Probit analysis using SPSS 16 for windows. The percentage of cell viability of each concentration of the sample obtained is calculated using the formula:

$$\% \text{ Viability} = \frac{\text{absorbance sample} - \text{absorbance control media}}{\text{absorbance control cell} - \text{absorbance control media}} \times 100$$

Phytochemical compound analysis

The analysis of phytochemical is conducted with Thin Layer Chromatography (TLC). The analysis is conducted to obtain the metabolite secondary information from each extract understorey plant. The extract used in this test is only the extract with IC_{50} value less than 70 of µg/mL. The phytochemical compound analysis includes alkaloid, steroid, terpenoids, phenol, flavonoid and tannin.

RESULTS AND DISCUSSIONS

Results

Inventory of understorey plants in the National Park of Gunung Gede Pangrango (TNGGP) in Zone of Cibodas Resort is conducted using the transect plot. The results found that there are 97 understorey plants and they consisted of 52 families. The dominance family consisted of 12 families as follows: Asteraceae (8 species), Solanaceae (6 species), Zingiberaceae (4 species), Moraceae (4 species), Melastomataceae (4 species) and Acanthaceae (3 species), Begoniaceae (3 species), Gesneriaceae (3 species), Malvaceae (3 species), Myrsinaceae (3 species), Piperaceae (3 species), and Polygalaceae (3 species). The other 40 families only consisted of one species plant. The families of all understorey plants from TNGGP are shown in Figure 1.

All the understorey plants then being evaluated for their potentials as the anticancer cervixs agents using HeLa cells. The IC_{50} value of 97 understorey plants are shown in Table 1.

The results show that from the 97 plants tested, there are five potential anticancer plants for HeLa cell cancer i.e. *Physalis peruviana* L., *Tithonia diversifolia* (Hemsl.) A. Gray, *Lantana camara* L., *Clidemia hirta* (L.) D.Don, and *Solanum torvum* Sw. The specifications of each understorey plants are shown in Table 2.

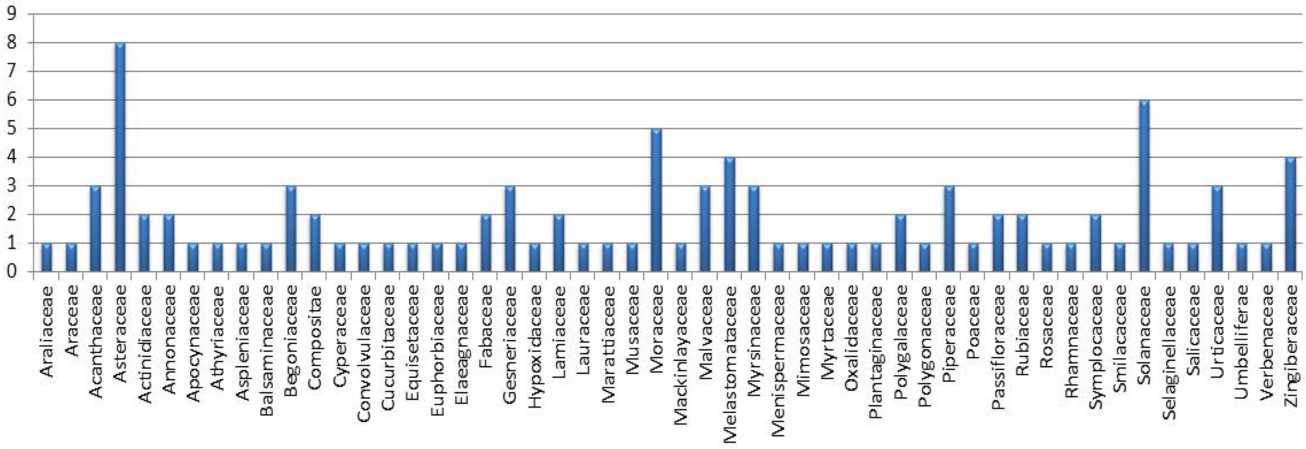

Figure 1. Family of understorey plants from the National Park of Gunung Gede Pangrango, West Java, Indonesia

Table 1. IC_{50} value of 97 understorey plants from the National Park of Gunung Gede Pangrango (West Java, Indonesia) against HeLa cell cancer

Species	Family	Local name	IC_{50} value (µg/mL)	Traditional medicine uses*
Agalmyla parasitica (Lam.) Kuntze	Gesneriaceae	Kitando	269.39	Anti-pyretic
Ageratum conyzoides L.	Asteraceae	Babadotan	121.22	Dyspepsia syndrome, stop the bleeding
Amomum pseudofoetens Valeton.	Zingiberaceae	Tepus sigung	74.83*	Contusions
Angiopteris evecta (G.Forst.)Hoffm	Marattiaceae	Paku kebo	582.77	Hair nutrient
Anotis hirsuta (L.f.) Boerl	Rubiaceae	Kasimukan	371.65	Fever
Ardisia fuliginosa Bl.	Myrsinaceae	Kiajag	158.13	Antipirurytic
Artemisia vulgaris L.	Asteraceae	Rokat mala	205.96	Gout, fluor albus
Asplenium nidus L.	Aspleniaceae	Kadaka	>1000	Fever
Austroeupatorium inulifolium (Kunth).King & Robinson	Asteraceae	Kirinjuh	162.82	Treatment of wound
Begonia isoptera Dryand.	Begoniaceae	Hariang bodas	473.58	Anti-pyretic
Begonia robusta Bl.	Begoniaceae	Hariang beureum	>1000	Anti-pyretic
Begonia sp.	Begoniaceae	Haring tangkal	>1000	Anti-pyretic
Bidens pilosa L.	Asteraceae	Hareuga/ketul	190.13	Influenza, sore throat
Blumea balsamifera (L) DC.	Asteraceae	Sembung gunung	352.13	Antihelmintic agent
Brassaiopsis glomerulata	Araliaceae	Panggang cucuk	576.43	Kidney
Brugmansia suaveolens Bercht & C Presl.	Solanaceae	Kucubung	497.98	Anti-diabetic
Bryonopsis laciniosa (L.) Naudin	Cucurbitaceae	Koreh kotok	>1000	Anti-diabetic
Carex baccans Nees.	Cyperaceae	Rumput teki	620.71	Anti-Influenza
Centella asiatica (L.) Urb.	Apiaceae	Pegagan	278.05	Anti-itching
Cestrum aurantiacum Lindley	Solanaceae	Kijogo	>1000	Anti-itching
Cestrum elegans (Brongniart ex Neumann) Schlechtendal.	Solanaceae	Jawerkotok leuweung	173.21	Anticancer
Clidemia hirta (L).D.Don	Melastomataceae	Harendong bulu	37.18	Anticancer
Coleus galeatus (Vahl) Benth.	Lamiaceae	Congkok	>1000	Antipirurytic
Curculigo capitulata (Lour.) Herb.	Cyperaceae	Congkok	460.40	Anti-Oxidan
Cyrtandra picta Bl.	Gesneriaceae	Reundeu badak	>1000	Antipyretics
Cyrtandra populifolia Miq.	Gesneriaceae	Reundeu beureum	501.00	Analgesic
Debregeasia longifolia (Burm.f.) Wedd.	Urticaceae	Totongoan	>1000	Analgesic
Diplazium repandum Blume.	Athyriaceae	Paku buah	574.37	Anti-dysentery
Elaeagnus latifolia L.	Elaeagnaceae	Kicepot/kakaduan	389.36	Caring for miss V
Embelia pergamacea A.DC.	Myrsinaceae	Kicemang gede	616.31	Mouthwash
Embelia ribes Burm.f.	Myrsinaceae	Kicemang beurit	429.99	Sprue
Equisetum debile Roxb. ex Vaucher.	Equisetaceae	Paku ekor kuda	343.93	Antipirurytic
Euchresta horsfieldii Benn.	Leguminosae	Kijiwo	125.71	Anti-rheumatic
Eupatorium riparium Regel.	Asteraceae	Teklan	151.45	A Treatment of wound
Eupatorium sordidum L.	Asteraceae	Babakoan	>1000	A Treatment of wound

Ficus laevigata Vahl.	Moraceae	Kihamplas	322.22	Stronger and antibody
Ficus lepicarpa Blume	Moraceae	Bisoro	191.16	Anti-diarrhea
Ficus obscura var. Borneensis.	Moraceae	Kihamplas besar	349.04	Analgesic
Ficus ribes Bl.	Moraceae	Walen	>1000	Analgesic
Ficus variegata Blume	Moraceae	Kondang benying	349.87	Anti-diarrhea
Flacourtia rukam Zoll.	Flacourtiaceae	Rukem	422.46	Anti-diarrhea
Gynura aurantiaca (Blume) DC.	Asteraceae	Santoloyo/sintrong	103.64	Dyspepsia Syndrome
Hedychium roxburghii Bl.	Zingiberaceae	Gandasoli	636.02	Analgesic
Homalanthus populneus (Geiseler) Pax	Euphorbiaceae	Kareumbi	450.67	Antipirurytic
Impatiens platypetala Lindl.	Balsaminaceae	Pacar tere	970.92	Skin disease
Imperata cylindrica (L.) P.Beauv.	Poaceae	Eurih/alang-alang	756.21	Fever
Lantana camara L.	Verbenaceae	Saliara/stekan	43.17*	Anticancer
Lasianthus purpureus Bl.	Rubiaceae	Kahitutan tangkal	825.77	Fever
Litsea cubeba (Lour.) Pers.	Lauraceae	Kilemo	682.12	Fever
Medinilla speciosa Reinw.ex Blume	Melastomataceae	Areuy boboledan	465.34	Antipirurytic
Medinilla verrucosa Bl.	Melastomataceae	Harendong bokor	237.31	Analgesic
Melastoma setigerum Bl.	Melastomataceae	Harendong koneng	583.78	Analgesic
Merremia umbellata (L.) Hallier.f.	Convolvulaceae	Harendong lalaki	142.85	Analgesic
Montanoa sp.	Asteraceae	Kidabo	135.45	Analgesic
Musa acuminata Colla.	Musaceae	Pisang kole	>1000	Skin disease
Mussaenda frondosa L.	Rubiaceae	Kingkilaban	258.74	Antipirurytic
Orophea hexandra Bl.	Annonaceae	Kisauheun	142.94	Anti-diabetic
Oxalis intermedia A. Rich.	Oxalidaceae	Calincing gede	141.85	Anti-hypertency
Passiflora edulis Sims.	Passifloraceae	Pasi	>1000	Fever
Passiflora suberosa L.	Passifloraceae	Konyal	845.69	Kidney
Physalis peruviana L.	Solanaceae	Cecenet/ciplukan	67.86*	Anticancer
Pilea trinervia (Roxb.)Wight.	Urticaceae	Seureuh leuweung	>1000	Cough
Piper arcuatum C.Presl.	Piperaceae	Rindu leutik	177.87	Mouthwash
Piper baccatum Bl.	Piperaceae	Seureuh kandel	273.08	Bad breath
Piper sp.	Piperaceae	Haruman	218.93	Antipirurytic
Pithecellobium clypearia (Jack) Benth	Fabaceae	Ki haruman	124.14	Antipirurytic for animal
Plantago major L.	Plantaginaceae	Ki urat	359.69	Anti-diabetic
Polyalthia subcordata Bl.	Annonaceae	Nona Leuweng	>1000	Anti-itching
Polygala paniculata L.	Polygalaceae	Akar wangi	109.23	Eczema
Polygala venenosa Juss.ex Poir.	Polygalaceae	Katutungkul	747.15	Cough
Polygonum chinense L.	Polygonaceae	Tiwu bungbum	789.04	Teeth disease
Rauwolfia javanica L.	Apocynaceae	Lame	577.41	Antifungal
Rubus sundaicus Bl.	Rosaceae	Hareu'eus	509.54	Kidney disease
Sanicula elata Ham.ex D.Don.	Apiaceae	Seladri gunung	818.79	Fever
Saurauia blumiana Benn.	Actinidiaceae	Ki leho canting	>1000	Fever
Saurauia pendula Bl.	Actinidiaceae	Jawer kotok	492.95	Antipirurytic
Schismatoglottis calyptrata (Roxb.) Zoll.	Araceae	Cariang	399.00	Contusion
Scutellaria discolor Colebr.	Lamiaceae	Antanan/pegagan	482.09	Anti-diabetic
Selaginella opaca Warb.	Selaginellaceae	Paku rane	292.11	Skin
Sida rhombifolia L.	Malvaceae	Sanagori/sidaguri	269.97	Skin
Smilax macrocarpa Bl.	Smilaceae	Canar	931.51	Shipilis
Solanum torvum Sw.	Solanaceae	Tekokak	58.96*	Anticancer
Solanum verbascifolium L.	Solanaceae	Teter	371.53	Bio pesticide
Stephania venosa (Blume) Spreng.	Menispermaceae	Geureung bodas	164.54	Anticancer
Sterculia longifolia Vent.	Sterculiaceae	Palahlar gede	399.48	Fever
Strobilanthes blumei Bremek	Acanthaceae	Bubukuan gede	868.50	Kidney
Strobilanthes cernua Bl.	Acanthaceae	Bubukuan kembang bidas	968.26	Kidney
Strobilanthes filiformis Bl.	Acanthaceae	Bubukuan letik	>1000	Kidney
Symplocos fasciculata (Kuntze) Zoll.	Symplocaceae	Jirak leutik	469.83	Antifungal
Symplocos javanica (Blume) Kurtz.	Symplocaceae	Jirak	296.20	Antifungal
Syzygium zeylanicum L. (DC.)	Myrtaceae	Kijambaga	204.05	Antiseptic
Tithonia diversifolia (Hemsl.) A. Gray	Asteraceae	Rigow	<32.50*	Anticancer, diabetic
Trevesia sundaica Miq.	Araliaceae	Panggang rante	266.30	Anti-hypotency
Urena lobata L.	Malvaceae	Pungpurutan	609.61	Anti-rheumatic
Villebrunea rubescens Bl.	Urticaceae	Nangsi	851.98	Cough
Zingiber inflexum Bl.	Zingiberaceae	Tongtak leutik	192.27	Analgesic
Zingiber odoriferum Bl.	Zingiberaceae	Tongtak	>1000	Anti-asthmatic

Note: IC_{50}< 100 µg/mL = active moderate active, 100 µg/mL < IC_{50}< 1000 µg/mL = moderate active, IC_{50}> 1000 µg/mL= non toxic (Prayong et al. 2008)

Table 2. Phytochemical compounds and cytotoxic activity againts hela cervixs cancer cells of understorey plants from the National Park of Gunung Gede Pangrango, West Java, Indonesia

Plant name	Family	Local name	IC_{50} (μg/mL)	Phytochemical compound
Tithonia diversifolia (Hemsl.) A. Gray.	Asteraceae	Rigow	3.38	Alkaloid, phenolics, steroid, terpenoids
Clidemia hirta (L). D.Don	Melastomataceae	Harendong bulu	36.92	Alkaloid, phenolics, flavonoids, steroid, serpenoids, tanin
Lantana camara L.	Verbenaceae	Saliara/stekan	43.54	Alkaloid, phenolics, flavonoids, steroid, terpenoids
Solanum torvum Sw.	Solanaceae	Tekokak	59.08	Alkaloid, phenolics, flavonoids, steroid, terpenoids
Physalis peruviana L.	Solanaceae	Cecenetan	67.85	Alkaloid, phenolics, flavonoids, steroid, terpenoids

Figure 2. Understorey plants that potential as cervixs anticancer from the National Park of Gunung Gede Pangrango, West Java, Indonesia. A. *Physalis peruviana* L., B. *Tithonia diversifolia* (Hemsl.) A. Gray, C. *Lantana camara* L, D. *Clidemia hirta* (L) D.Don, E. *Solanum torvum* Sw.

Discussions

The inventories of the understorey plants in Cibodas Resort, National Park of Gunung Gede Pangrango, find 97 understorey plants. The families of understorey plants consisted of 52 families, and the highest family is Asteraceae consisting of eight (8) species. Indonesia has a huge biodiversity and a prestigious heritage on usage of herbal medicine. The research of Yusro et al. (2014) states from the inventories of the medicinal plants are used for the fever disease in 151 Dayak sub-ethnics, including Dayak Iban, Kanayatn, Bukat and Daro' in West Kalimantan, there are 33 species consisting of 19 families. The dominant family used to overcome the fever disease is Rubiaceae, and the most frequently used parts are the leaves with the boiling process and drink. Diba et al. (2013) find 68 species of plants used for the medicine in Dayaknese communities in West Kalimantan. The plants are used to overcome the stomach ache, headache, injury, acne, menstrual cramps, fever, cough and influenza, bone injury, and malaria fever.

Medicinal plants have a large amount of chemical components, and the extraction of drug candidates from the plants needs a proper selection of extraction and screening method. This is due to find the bioactive compound from the plants. In this research, the leaf from the understorey plant is the extraction with methanol. Methanol extract has a high cytotoxicity than other solvents. In line with our results, Alzeer et al. (2014) investigate the impact of variable solvent extraction techniques of Palestine medicinal plants and cytotoxic effects to HeLa cells, B16F10 and MCF-7. The solvent consists of acetone, apple vinegar, 90% ethanol, coconut water, 80% methanol, and grape vinegar or 5% acetic acid. The results show that the highest cytotoxicity value is achieved from ethanol and methanol solvents. Yuenyongsawad et al. (2014) also use ethanol as a solvent for extraction of *Cassia garrettiana* heartwood. The extract is used to inhibit cancer cell as follow: HeLa cells, MCF-7, KB cells and HT-29. The extract ethanol, *C. garretiana*, consists of five compounds i.e. chrysophanol, piceatannol, aloe-emodin, emodin and cassigarol E. The results show that only four compounds have anti-cancer agents, as follow, the emodin has the highest activity against HeLa cells with IC_{50} 0.82 μg/mL, the aloe emodin has the highest activity against HT-29 with IC_{50} 0.29 μg/mL, the chrysophanol has the highest activity against KB cells with IC_{50} 0.045 μg/mL, and the cassigarol E has the highest activity against MCF-7 with IC_{50} 0.021 μg/mL.

The need for utilizing the herbal medicine to overcome cancer, especially the cervical cancer because the cervical cancer in Indonesia is the first rank as the deadly cancer on women (Azis 2009), and this cancer is also the third of most common cancer in Iran (Sadjadi et al. 2005). Treatments of cervical cancer usually consist of chemotherapy, radiotherapy and surgery, but these treatments still have the side effect to the patients. Lippert et al. (2008) state most of the drugs used for anti-cancer are expensive, highly toxic and also having side effect such as resistance mechanism. Therefore, many researchers conduct the research on used medicinal plants to overcome the cervical cancer.

Patel and Patel (2011) state that the plants for anti-cancer mostly have the bioactive compounds, consisting of flavonoid, phenolic acids and phenolic diterpenes. Similar to this statement, the phytochemical screening of understorey plants from the National Park of Gunung Gede

Pangrango found that the major bioactive compounds are flavonoids, alkaloids, phenol, terpenoids, and steroid (Table 2). Flavonoids are a group of aldehyde and it is commonly used for medicine. Chandrappa et al. (2014) found that flavonoid from *Carmona retusa* (Vahl.) Masam has anticancer activity. Alkaloid is found on *Physalis peruviana, Tithonia diversifolia, Lantana camara, Clidemia hirta,* and *Solanum torvum.* This compound has a function as anti-cancer. Li et al. (2008) revealed that alkaloid isolated from *Angelica dahurica* inhibit HeLa cell growth. The alkaloid also induced the apoptosis in HeLa cells, suppressed the tumor growth, and interfered the cell cycle progression.

Prayong et al. (2008) have classified the substrates based on the cytotoxicity, and the selectivity is based on five categories i.e. active (IC_{50}< 100 μg/mL), moderate active (100 μg/mL < IC_{50}< 1000 μg/mL), non-toxic (IC_{50}> 1000 μg/mL). The five understorey plants from the National Park of Gunung Gede Pangrango are categorized on the first category which is active, because all the IC_{50} values are <100 μg/mL. Five of six understorey plants that have cytotoxic activity chosen to further study. The plants included in *Physalis peruviana* L., *Tithonia diversifolia* (Hemsl.) A. Gray., *Lantana camara* L., *Clidemia hirta* (L.) D. Donn., and *Solanum torvum* Sw., with IC_{50} value are 67.85 μg/mL, 3.38 μg/mL, 43.54 μg/mL, 36.93 μg/mL, and 59.09 μg/mL, respectively.

Patel et al. (2009) found out that the extract of *Solanum nigrum* can inhibit HeLa cell line from in-vitro cytotoxicity test. *Solanum torvum* and *Solanum nigrum* are the plants species from the family of Solanaceae. Arjomandnejad et al. (2014) state HeLa cells on the xenograft model as a comprehensive description for in vivo investigation. Other researchers, Venkateswarlu et al. (2015) have extracted *Cynodon dactylon* with petroleum ether, and these compounds are toxic to HEP-2, HeLa and MCF-7 cells. The value of IC_{50} is recorded at a concentration of 0.156 mg/ml – 0.625 mg/ml of petroleum ether extract of *Cynodon dactylon.* Meanwhile, Larasati et al. (2014) use the cinnamon essential oil isolated from *Cinnamomum burmannii* as co-chemotherapeutic agent of cisplatin against HeLa cells. The result shows that the cinnamon essential oil has cytotoxic effect on HeLa cells with IC_{50} value of 250 μg/mL, while the cisplatin shows the cytotoxic effect with IC_{50} value of 18 μM. HeLa cells are used in the evaluation anticancer from the herbal medicine. Further research will be conducted to know the compound and the mechanism of anticancer from each five understorey plant.

In conclusion, understorey plants in the National Park of Gunung Gede Pangrango consist of 97 plants and are divided into 52 families. The dominant families consist of Asteraceae, Solanaceae, Moraceae, Melastomataceae, Zingiberaceae, Acanthaceae, Begoniaceae, Gesneriaceae, Malvaceae, Myrsinaceae, Piperaceae and Urticaceae. Five potential understorey plants as the anticancer cervixs are *Physalis peruviana, Tithonia diversifolia, Lantana camara, Clidemia hirta,* and *Solanum torvum* with IC_{50} values to be 67.85μg/mL, 3.38 μg/ mL, 43.54 μg/ mL, 36.93 μg/ mL, and 59.09 μg/ mL respectively. The five understorey plants from the National Park of Gunung Gede Pangrango are categorized on the first category which is active value, because all of the IC_{50} values are <100 μg/mL.

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