Antiproliferative Activity of the *Eupatorium riparium* Reg. Leaves Washbenzine Extract: *In vitro* Study on HeLa Cell Line

Aktivitas Antiproliferatif Ekstrak Washensin Daun *Eupatorium riparium* Reg. : Studi *In Vitro* Pada HeLa Cell line

Linus Yhani Chrysomo¹ *, L. Hartanto Nugroho², Subagus Wahyuono³, Aditya Krishar Karim¹, Kumiko Terada⁴, and Tsutomu Nohno⁴

¹Department of Biology, Faculty of Science and Mathematic, Universitas Cenderawasih, Papua, Indonesia
²Department of Biology, Faculty of Biology, UGM Yogyakarta, Indonesia
³Department of Pharmacy Biology, Faculty of Pharmacy, UGM Yogyakarta, Indonesia
⁴Department Molecule and Development Biology, Kawasaki Medical School, Jepang
E-mail address: chrysyanka@yahoo.com *Corresponding author

Abstract

*Eupatorium riparium* Reg. is medicinally important plant, native of Mexico and the west Indies, introduced in Java long ago. This plant has a history of use for traditional medicine of various cultures world wide and is commonly used to treat hypertension, systole heart failure, diuretics, anticancer, antifungal and bacterial diseases. Study on the HeLa cell line was arranged for a month. This study was aimed to investigate the possible antiproliferative activity of washbenzine extract of *E. riparium* leaves against human cervical cancer (HeLa) cell line. Antiproliferative activity was measured by cell proliferation reagents WST-1, and test for 1, 2, and 4h after incubated for 72h, at 37°C with 5%CO₂. The result showed that the washbenzine extract of *E. riparium* leaves possessed potential antiproliferative activity against HeLa cell lines with IC₅₀ values of 102.69 µg/ml (1h), 198.67 µg/ml (2h), respectively. Further study is suggested to understand anticancer mechanism on HeLa cell line.

Keywords: *Eupatorium riparium* Reg, antiproliferative, HeLa, WST-1

Abstrak

*Eupatorium riparium* Reg. adalah tumbuhan obat penting asli dari Mexico dan India Barat, yang masuk ke tanah Jawa sejak tahun 1800. Tumbuhan ini mempunyai catatan sejarah digunakan untuk obat tradisional dalam berbagai kultur budaya bangsa secara luas di seluruh dunia dan biasa digunakan untuk obat hipertensi, gatal jantung, diuretik, antikanker, antifungi, dan penyakit yang disebabkan oleh bakteri. Studi pada HeLa cell line ini dilakukan selama satu bulan. Selanjutnya, studi ini bertujuan meneliti aktivitas antiproliferatif ekstrak washensin daun *E. riparium* terhadap kanker servik manusia HeLa cell line. Aktivitas antiproliferatif diuji menggunakan reagen proliferatif sel WST-1 dengan waktu 1, 2, dan 4 jam setelah diinkubasi selama 72 jam pada suhu 37°C dan 5%CO₂. Hasil penelitian menunjukkan bahwa ekstrak washensin daun *E. riparium* mempunyai aktivitas proliferatif yang potensial terhadap HeLa cell line dengan nilai IC₅₀ berikut 102.69 µg/ml (1 jam), 198.67 µg/ml (2 jam). Saran selanjutnya, penelitian lanjutan perlu dilakukan untuk mengetahui mekanisme antikanker HeLa cell line.

Kata kunci: *Eupatorium riparium* Reg, antiproliferative, HeLa, WST-1

Diterima: 28 Juli 2012, disetujui: 12 November 2012

Introduction

Cervical cancer is an important health problem worldwide, being the second most common cancer among women and first ranking in many developing countries (Rock *et al.*, 2000). A large number of the plants are claimed to possess the antimalaria, anticancer, antimicrobials, antifungal, antiinflammatory, antibiotic properties in the traditional healing
system and this system are used extensively by the tribal people worldwide (Ananil et al., 2000; Aranda et al., 2011).

Natural products have long been used to prevent and to treat many diseases, including cancer and thus they are good candidates for the development of anti-cancer drugs (Smith-Warner et al., 2000 in Goncalves et al., 2010). Plant derived agents are being used for the treatment of cancer. Natural and some synthetic compounds can prevent, suppress, or reverse the progression of cancer. Several studies have demonstrated that extracts from several herbal medicines or mixtures had an anticancer potential in vitro or in vivo (Bonham et al., 2002; Lee et al., 2002; Madhuri and Pandey, 2009).

Traditional healing systems have become an increasing interest as many people believe that they can be used without any risk and side effects (Roeder and Wiedenfeld, 2011). World Health Organization (WHO) reported that in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs (Misra, 2009).

_Eupatorium riparium_ Reg. is a shrub annual plant grown as weed in the valley and river banks, belongs to the family Asteraceae. This plant is medicinally important plant, native of Mexico and the west Indies, long ago before in 1800 introduced to Java, and at present naturalized in many places on Mt. Gede, Mt. Pangragano at 1000–2500 sea level (Bockor and van den Brink, 1968).

_Eupatorium_ genus has been used for medicinal properties for many decades. A number of bioactive natural products have been reported in extracts of _Eupatorium_ spp. as a promising bioresource for preparation of drugs and value-added products (Sharma et al., 1998). A decoction of _E. riparium_ leaves is taken to treat cardiac palpitations, asthma, and gastritis (Fortin et al., 2003 in Roeder and Wiedenfeld, 2011).

The alkaloid extracts from _E. riparium_ and _E. adenophorum_ were tested against _Escherichia coli_, a Gram negative bacterium as effective as the commercial antibiotic synthetic Imipinem. Seemingly, the alkaloid extracts were bactericidal in activity (Rosuman and Lirio, 2008).

Chrysotomo et al., (2011) showed that the methylyripapichromene-A from _E. riparium_ was only founded on leaves, and the highest of methylyripapichromene-A by was benzine extract of _E. riparium_ leaves from Mt. Menoreh at Samigaluh, compared from Tawangmangu Karanganyar and Mt. Merapi in Kaliurang. Fakhrudin (2006) isolated methylyripapichromene-A from chloroform extract of _E. riparium_ and it had cytotoxic activity against HeLa and Vero cell line.

Chrysotomo et al., (2011) reported that benzine extract of _E. riparium_ leaves from Mt. Menoreh at Samigaluh possessed potential cytotoxicity activity against 293A cancer cell line with IC_{50} values of 76.22 μg/ml (1h), 79.27 μg/ml (2h) and 91.52 μg/ml (4h) and HCT-116 cancer cell line with IC_{50} values of 290.59 μg/ml (1h), 260.99 μg/ml (2h) and 203.75 μg/ml, respectively.

Another studies reported that _E. riparium_ have been alelopathy effect towards population of weeds Galinsoga ciliata Raf. and Galinsoga parvisflora Cav. The water extract of _E. riparium_ can inhibited the germination of seeds and it inhibits the growth of radicule and plumule of Galinsoga ciliata Raf. and Galinsoga parvisflora Cav. (Kunwar 2003; Rai and Tripathi, 2005).

Yunita et al., (2009) showed that leaf extract of _E. riparium_ contained saponin, tannin, quinon and steroid, and this plant showed cytotoxic on A. aegypti larvae and significant effect on percentage of pupae's development. The purpose of this study was to examine the antiproliferative activity of wasbenzine extract of _E. riparium_ leaves from Mt. Menoreh in Samigaluh, against HeLa cancer cell line.

**Materials and Method**

**Preparation of _E. riparium_ was benzine extract**

_Eupatorium riparium_ Reg. plant was collected from Mt. Menoreh in Samigaluh, The plant was identified by Taxonomy Laboratorium, Gadjah Mada University. The leaves were washed, dried and chopped finely using a blender. Dried material (100 g) were
exhaustively extracted with wasbenzine maceration. The wasbenzine extract was filtered and concentrated using a rotary evaporator, evaporation to dryness.

Preparation of cell line

HeLa cell line (human cervical adenocarcinoma cell line) was obtained from Laboratory stock of Oncology Laboratory, Department of Molecular and Developmental Biology, Kawasaki Medical School, Japan. HeLa cell line was grown on Dulbecco's Modified Eagle Media (DMEM, Sigma) containing 10% v/v Fetal Bovine Serum (FBS) (Sigma) and 1% v/v kanamycin (Sigma). The cultures were maintained at 37°C in humidified atmosphere of 5% CO₂.

In vitro assay for antiproliferative activity

The cell suspension 4.0x10⁵ cell/ml (100μl) was plated into 96 well microplate (Nunc, Germany) and was treated with different concentration of wasbenzine extract isolated from E. riparium leaves, in a serial dilution (500, 250, 125, 62.5, 31.25 dan 15.625, 7.8125 μg/ml). Following treatment, plates were incubated in CO₂ incubator at 37°C for 72 h. Medium was removed by aspirator and added with 10 μl cell proliferation reagents WST-1 for 1 h, 2 h and 4 h and incubated in CO₂ incubator at 37°C. The absorbance was read at wavelength of 450 nm using ELISA reader type Varioskan Flash (Thermo scientific). The percentage cellular viability was calculated with appropriate control taken into account. The concentration which inhibited 50% of cellular growth (IC₅₀) was determined. The inhibitory rate of cell proliferation was calculated by the following formula:

\[
\text{Growth (\%)} = \frac{\text{OD Control} - \text{OD treated}}{\text{OD Control}} \times 100
\]

Results and Discussion

In this study, toxicity data were expressed as IC₅₀, a concentration of extracts that cause 50% inhibition of survival cell and was obtained by plotting the percentage survival cell versus concentration of wasbenzine extract samples (Francoeur and Assalian, 1996). The study used concentration range of wasbenzine extract of E. riparium leaves 0–500 μg/ml, because of the extract that gave an IC₅₀ value of 1000 μg/ml or less was considered chemopreventive activities (Doyle and Griffiths, 2000).

The results showed that the wasbenzine extract of E. riparium leaves possessed antiproliferative effect against cell HeLa cell line with IC₅₀ values of 102.69 μg/ml (1h), 198.67 μg/ml (2h), respectively. It indicated that wasbenzine extract of E. riparium leaves possessed potential antiproliferative against HeLa cell line. The cytotoxicity result indicated time and dose dependent concentration of the extract (Figure 1).

The activities of these extract against HeLa cell line might be due to the presence of highly complex compounds that present in E. riparium. Different compounds might influence different biochemical processes or stages in different manners.

Several studies have reported that Genus Eupatorium contains sesquiterpene lactones, diterpene lactones, flavonoid, terpenoid and sterol. Sesquiterpene lactones and diterpene lactones showed cytotoxic activity on cell lines (Suksamran et al., 2004; Huo et al., 2004; Shen et al., 2005; Zhang et al., 2008).

Sesquiterpene lactones and diterpene lactones isolated from E. kirinense showed cytotoxic activity on HeLa cell line (Shen et al., 2005) and eupanulia C isolated from E. liddleyanum had cytotoxic activity against p-338 and A-549 cell line (Huo et al., 2004).

Chemical compounds composition of Chromolaena odorata (synonym; E. odoratum) extract contained flavonoids, saponins, tannins and steroids, and C. odorata extracts revealed antibacterial activities, inhibiting the growth of Bacillus subtilis, Staphylococcus aureus, and Salmonella typhimurium and this report also showed that the extract of C. odorata could reduce the number of parasites Trichomonas vaginalis and Blastocystis hominis (Vital and Rivera, 2009).

Fakhrudin (2006) showed that the methylripariochromene-A isolated from chloroform extract of E. riparium had cytotoxic activity toward Hela and Vero cell line with
IC₅₀ of 58.32 µg/ml, and 80.95 µg/ml, respectively. Other studies have reported that methylripariochromene-A had antifungal activity (Sharma et al., 1998). Methylripariochromene-A isolated from of E. riparium has antifungal activity towards pathogenic fungi Colletotrichum gloeosporioides (Bandara et al., 1992). Studied by Hidayat (2002) reported that hexane extract of E. tripinerve had cytotoxic activity against myeloma cell line with ED₅₀ of 5.85 µg/ml using Brine Shrimp Lethality Test. Therefore, the presence of the methylripariochromene-A, sesquiterpene lactones, diterpene lactones, flavonoid, terpenoid and sterol could be assumed to be responsible for the antiproliferative activities of wasbenzine extract of E. riparium in this study.

Several studies have reported many compounds from herbal or compounds from extract have different cytotoxicity activity on the different cell line. Arkadiusz et al., (2001) reported that quercetin and DMSO modulated and changed Bcl-2 gene expression (Apoptosis regulating proteins) during myogenogenesis on C2C12 cell line. Other study by Meiayoto and Septisetyani (2005) reported that fraction of ethanolic extract of Sambung Nyawa (Gynura procumbens (Lour.) Merr. XIX-XX which was selected to represent the relatively polar fraction and contained phenolic compound had the antiproliferative and apoptotic effect against HeLa cell line with IC₅₀ of 119µg/ml.

Cell viability profiles which was produced from MTT assay showed that methanolic extract and the other fractions (n-hexane, methylene chloride, ethyl acetate and methanol) from Pauh Kijang bark extract decreased HeLa cell viability compare to control cells in the concentration dependent manner with IC₅₀ of 59, 92, 30, 22, and 33 µg/ml, respectively and the strongest cytotoxic activity was showed by ethyl acetate fraction (Kusharyanti et al., 2008).

Shao et al., (2005) reported that Arsenic trioxide (As₂O₃) was a major ingredient of traditional Chinese medicine showed cytotoxic activity using MTT assay and induce apoptosis of human gastric carcinoma cells (MKN45), indicated by the presence of cell shrinkage, membrane blebbing, fragmentation of nuclei, and formation of apoptotic bodies on MKN45. Another study by Yu et al., (2006) showed that antitumor effect of Chinese compound Jinlongshe (JLS) granules were on sarcoma 180 and MKN-45 human gastric cancer cell lines in vivo.

![Figure 1. Correlation survival cell (%) of HeLa cell line with concentration of wasbenzine extract of E. riparium leaves (µg/ml) after incubation for 72h at 37°C with 5% CO₂, and after add WST-1 reagent for 1h (α-brown), 2h (△-blue).](image-url)
This study showed that the ability of wasabenine extract to inhibit proliferation of HeLa cell line was estimated by analysing its effect on the growth of the cells. The growth of the untreated (control) and treated cell line after incubation for 72 h was photographed using a phase contrast microscope (data not shown). In the untreated cells after 72 h incubation, cells are growing normally as indicated by the presence of formazan dye formed. The more the extract concentration increases, the more formazan dye decreases. The formation of formazan dye directly correlates to the number of metabolically active cell in the culture (Francouer and Assalian, 1996). It is indicated that wasabenine extract of E. riparium leaves proved to possess antiproliferative properties against HeLa cell line tested. Therefore, it may have potential as a chemotherapeutic agent since it has IC50 values less than 1000 μg/ml (Doyle and Griffiths, 2000). Further investigation is suggested to know about inhibitory mechanism on HeLa cell line.

Conclusion

The wasabenine extract of E. riparium leaves possessed potential antiproliferative activity against HeLa cell line. This plant has potential as anticancer agent.

Acknowledgment

That was supported grant from Sandwich Program 2010, DIKTI Indonesia, and we also give our thanks to The Head of Department of Molecular and Developmental Biology, Kawasaki Medical School, Kurashiki, Japan for the grant of using laboratory facility.

References


