Biota Vol. 18 (1): 21–26, Februari 2013
ISSN 0853-8670

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

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

Linus Yhani Chrystomo[1*], L. Hartanto Nugroho[2], Subagus Wahyuono[3], Aditya Krishar Karim[1], Kumiko Terada[4], and Tsutomu Nohno[4]

[1]*Department of Biology, Faculty of Science and Mathematic, Universitas Cenderawasih, Papua, Indonesia*
[2]*Department of Biology, Faculty of Biology, UGM Yogyakarta, Indonesia*
[3]*Department of Pharmacy Biology, Faculty of Pharmacy, UGM Yogyakarta, Indonesia*
[4]*Department Moleculer and Development Biology, Kawasaki Medical School, Jepang*
*E-mail address: chrysyanka@ yahoo.com *Correponding 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 antiproliverative activity of wasbenzine 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%CO_2. The result showed that the wasbenzine extract of *E. riparium* leaves possessed potential antiproliferative activity against HeLa cell lines with IC_{50} 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, gagal 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 wasbensin 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%CO_2. Hasil penelitian menunjukkan bahwa ekstrak wasbensin daun *E. riparium* mempunyai aktivitas proliferatif yang potensial terhadap HeLa *cell line* dengan nilai IC_{50} 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, antiproliferatif, 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, antiinflamantory, 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. Pangrango at 1000–2500 sea level (Backer 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 palpations, 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).

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

Chrystomo *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 91.62 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 parviflora* Cav. The water extract of *E. riparium* can inhibited the germination of seeds and it inhibits the growth of radicule and plumulae of *Galinsoga ciliate* Raf. and *Galinsoga parviflora 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

Eupatoriun 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 maseration. 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 Moleculer 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 kanamicin (Sigma). The cultures were maintained at 37°C in humidified atmosphere of 5% CO_2.

***In vitro* assay for antiproliferative activity**

The cell suspension $4.0x10^3$ 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, 62.50, 31.25 dan 15.625, 7.8125 µg/ml). Following treatment, plates were incubated in CO_2 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 CO_2 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 (IC_{50}) was determined. The inhibitory rate of cell proliferation was calculated by the following formula:

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

Results and Discussion

In this study, toxicity data were expressed as IC_{50}, 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 IC_{50} value of 1000 µg/ml or less was considered chemoprenventif 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 IC_{50} values of 102.69 µg/ml (1h), 198.67 µg/ml (2h), respectively. It indicated that wasbenzine extract of *E. riparium* leaves possessed potentials antiproliferative against HeLa cell line. The cytotoxicity result indicated time and dose dependent concentration of the extract (Figure 1).

The activities of these extract againts 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. kiirunense* showed cytotoxic activity on HeLa cell line (Shen *et al.*, 2005) and eupanilia C isolated from *E. lidleyanum* 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

IC_{50} 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. triplinerve* had cytotoxic activity against myeloma cell line with ED_{50} 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 differents cell line. Arkadiusz *et al.*, (2001) reported that quercetin and DMSO modulated and changed Bcl-2 gene expression (Apoptosis regulating proteins) during myogenesis on C2C12 cell line. Other study by Meiyanto 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 IC_{50} 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 IC_{50} 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 (As_2O_3) 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*.

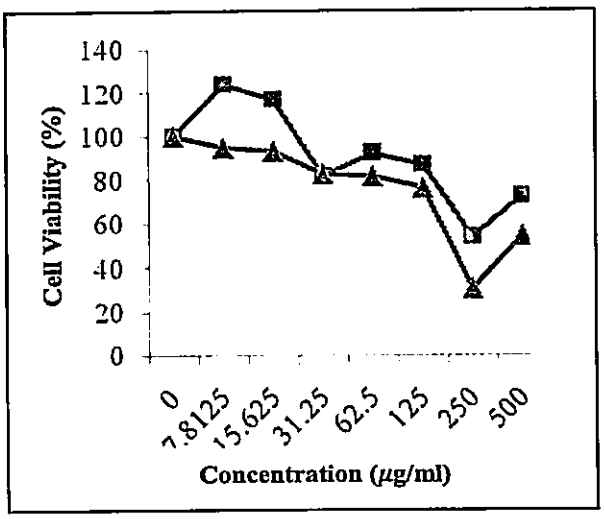

Figure 1. Correlation survival cell (%) of HeLa cell line with concentration of wasbenzine extract of *E. riparium* leaves (µg/ml) after incubation for 72h at 37°C with 5% CO_2. and after add WST-1 reagent for 1h (■-brown), 2h (▲-blue).

This study showed that the ability of wasbenzine 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 normaly 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 of cell in the culture (Francouer and Assalian, 1996). It is indicated that wasbenzine extract of *E. riparium* leaves proved to possess antiproliferative properties against HeLa cell line tested. Therefore, it may have potential as a chemotheraupetic agent since it has IC_{50} 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 wasbenzine extract of *E. riparium* leaves possessed potential antiproliferative activity againts 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 Molucular and Developmental Biology, Kawasaki Medical School, Kurashiki, Japan for the grant of using laboratory facility.

References

Ananil, K., Hudson, J.B., de Souzal, C., Akpaganal, K., Tower, G.H.N., Amason, J.T. and Gbeassor, M. 2000. Investigation of Medicinal Plants of Togo for Antiviral and Antimicrobial Activities. *Pharmacy Biological*, 38 (1): 40–45.

Aranda, R.S., Lopez, L.A.P., Arroyo, J.L., Garza, B.A.A. and de Torres, N.W. 2011. *Original Article: Antimicrobial and Antioxidant Activities of Plants from Northeast of Mexico*. Evidence-Based Complementary and Alternative Medicine. Hindawi Publishing Corporation, pp: 1–6.

Arkadiusz, O., Katarzyna, G., Wojciech, K. and Tomasz, M. 2001. Effect of Quercetin and DMSO on Skeletal Myogenesis from C_2C_{12} Skeletal Muscle Cells with Special Reference to PKB/Akt Activity, Myogenin and Bcl-2 Expression. *Basic Applied Myology*, 1: 31–44.

Backer, C.A. and van den Brink, R.C.B. 1968. *Flora of Java (Spermatophytes only)*. Volume 2.N.P.V. Noordhoff-Groningen the Netherlands, Published under the Auspices of the Ruksherbarium, Leyden. pp.362–378.

Bandara, B.M.R., Hewage, C.M., Karunaratne, V., Wannigama, G.P. and Adikaram, N.K.B. 1992. An Anti fungal Chromene from *Eupatorium riparium* Reg. *Journal Phytochemistry*, 31 (6): 1983–1985.

Bonham, M., Arnold, H., Montgomery, B. and Nelson, P.S. 2002. Molecular Effects of The Herbal Compound PC-SPES: Identification of Activity Pathways in Prostate Carcinoma. *Cancer Research*, 62: 3920–3924.

Chrystomo, L.Y., Karim, A.K., Terada, K. and Nohno, T. 2012. Evaluation of *In vitro* Antiproliferative Activity of *Eupatorium riparium* Reg. Leaves Benzene Extract Against 293A and HCT-116 Cancer Cell Lines. *Jurnal Bahan Alam Indonesia*, 1 (12): 1–7.

Chrystomo, L.Y., Sumardi, I., Hartanto, L.N. and Wahyuono, S. 2011. Penetapan Kadar Metilripariokromen-A pada Organ *Eupatorium riparium* Reg. dari Daerah yang Berbeda. *Jurnal Biota*, 16 (1): 107–113.

Doyle, A. and Griffiths, J.B. 2000. *Cell and Tissue Culture for Medical Research*. John Willey and Sons, Ltd. New York. 443 p.

Francouer, A.M. and Assalian, A. 1996. Microcat: A Novel Cell Proliferation and Cytotoxicity Assay Based on WST-1. *Journal Biochemistry*, 3: 19–25.

Fakhrudin, N. 2006. Skrining Senyawa Sitotoksik dari Tumbuhan Asal Taman Nasional Gunung Merapi Yogyakarta: Kajian Terhadap *Eupatorium riparium* Reg. (#04-KU-01-06). (*Thesis*). Fakultas Farmasi Universitas Gadjah Mada, Yogyakarta, 79 p.

Goncalves, S., Xavier, C., Costa, P., Albericio, F. and Rumano, A. 2010. Antioxidant, Cytotoxic and Apoptotic Activity of *Drosophyllum lusitanicum* Extracts. *Journal of Medicinal Plants Research*, 4 (15): 1601–1608.

Hidayat, M.A. 2002. Uji Aktivitas Ekstrak Heksan Daun *Eupatorium triplinerve* Vahl. Terhadap sel kanker Mieloma. *Jurnal Ilmu Dasar*, 3 (2): 92–97.

Huo, J., Yang, S.P., Ding, J. and Yue, J.M. 2004. Cytotoxic Sesquiterpen Lactones from *Eupatorium lindleyanum*. *Journal Natural Product*, 68 (1): 156–161.

Kunwar, R.P. 2003. Invasive Alien Plants and *Eupatorium*: Biodiversity and Livelihood Himalayan. *Journal Science*, 1 (2): 129–133.

Kusharyanti, I., Widiyantoro, A., Maryati and Meiyanto, E. 2008. Cytotoxic Activity of Pauh Kijang (*Irvingia malayana* Oliv.) Bark Extract. *Prosiding Konggres Ilmiah ISFI*. pp: 42–47.

Lee, S.M., Li, M.L., Tse, Y.C., Leung, S.C., Lee, M.M., Tsui, S.K., Fung, K.P., Lee, C.Y. and Waye, M.M. 2002. Paeoniae Radix, a Chinese herbal extract, Inhibits Hepatoma Cells Growth by Inducing Apoptosis in a p53 Independent Pathway.*Journal Life Science*,71: 2267–2277.

Madhuri, S. and Pandey, G. 2009. Some Anticancer Medicinal Plants of Foreign Origin. *Journal Current Scientific*, 96 (6): 779–783.

Misra, M. 2009. Editorial; Enhancement of Bioactive Compound Production, Antimicrobial Activity and Evaluation in Animal Models. *Journal Medic. Plants. Res.*, 3 (7): 1–3. Available online at *http://www.academicjournals.org/JMPR*.

Rai, J.P.N. and Tripathi, R.S. 2005. Allelopathy as a Factor Contributing to Dominace of *Eupatorium*. *Indian Journal Ecology*, 9: 14–20.

Rock, C.L., Micahael, C.W., Reynols, S.K. and Ruffin, M.T. 2000. Prevention of Cervix Cancer. *Review Oncology: Journal Hematology*, 33: 169–185.

Roeder, E. and Wiedenfeld, H. 2011. Pyrrolizidine Alkaloids in Plants Used in The Traditional Medicine of Madagascar and the Mascarene Islands. *Jornal Pharmazie*, 66: 637–647.

Rosuman, P.F. and Lirio, L.G. 2008. Alkaloids As Taxonomic Marker of Four Selected Species of *Eupatorium* Weeds. *Journal Research*, 16: 114–117.

Sharma, O.P., Dawra, R.K., Kurade, N.P. and Sharma, P.D. 1998. A Review of the Toxicosis and Biological Properties of Genus *Eupatorium*. *Journal Natural Toxins*, 6 (1): 1–14.

Suksamran, A., Chotiphong, A., Suavansri, T., Boongird, S., Timsuksai, P., Vimuttipong, S. and Chuaynugul, A. 2004. Antimycobacterial Activity and Cytotoxicity of Flavonoids From Flowers of *Chromolaena odorata*. *Archives Pharmacology Research*, 27 (5): 507–511.

Shen, Y.C., Lo, K.L., Kuo, Y.H. and Khail, A.T. 2005. Cytotoxic Sesquiterpen Lactones from *Eupatorium kiirunense*, A Coastal Plant of Taiwan. *Journal Natural Product*, 68: 745–750.

Shao, Q.S., Ye, Z.Y., Ling, Z.Q. and Ke, J.J. 2005. Cell Cycle Arrest and Apoptotic Cell Death in Cultured Human Gastric Carcinoma Cells Mediated by Arsenic Trioxide. *World Journal Gastroenterology*, 11 (22): 3451–3456.

Vital, P.G. and Rivera, W.L. 2009, Antimicrobial Activity and Cytotoxicity of *Chromolaena odorata* (L.f.) King and Robinson and *Uncaria perrottetii* (A.Rich) Merr. Extracts. *Journal Medicinal Plants Research*, 3 (7): 511–518.

Yu, Z.H., Wei, P.K., Xu, L., Qin, Z.F. and Shi, J. 2006. Anticancer Effect of Jinlongshe Granules on In Situ-Transplanted Human MKN-45 Gastric Cancer in Nude Mice and Xenografted Sarcoma 180 in Kunming Mice and Its Mechanism. *World Journal Gastroenterology*, 12 (18): 2890–2894.

Yunita, E.A., Suprapti, N.H. and Hidayat, J.W. 2009. Pengaruh Ekstrak daun Teklan (*Eupatorium riparium*) Terhadap Mortalitas dan Perkembangan Larva *Aedes aegypti*. *Journal Bioma*, 11 (1): 11–17.

Zhang, M.L., Wu, M., Zang, J.J., Irwin, D., Gu, Y.C. and Shi, Q.W. 2008. Review: Chemichal Contituents of Plant from The Genus Eupatorium. *Journal Chemical Biodiversity*, 5: 40–48.