Potential Cytotoxic on Breast Cancer Cells Line and Antioxidant of Water Extract of *Catharanthus roseus* [L] G.Don., *Dendropthoe petandra* L., *Curcuma mangga* Val., *Piper betle* L.

Wahyu Widowati[A*], Tjandrawati Mozef[B], Chandra Risdian[B], Hana Ratnawati[A], Susy Tjahjani[A], Ferry Sandra[C], Lusiana Darsono[A*], Sri Utami Sugeng[A*]

[A]Faculty of Medicine, Maranatha Christian University,
Jl. Prof drg. Suria Sumantri No.65, Bandung 40164, Indonesia
[B]Research Center for Chemistry, Indonesian Institute of Sciences,
Jl. Cisitu-Sangkuriang, Bandung 40135, Indonesia
[C]Department of Biochemistry and Molecular Biology,
Faculty of Dentistry, Trisakti University, Jakarta, Indonesia

*Corresponding author: wahyu_w60@yahoo.com /+6281910040010/+6222-2017621

Breast cancer is the most common cancer among women and the second leading cause of cancer deaths in women today. The madagascar prewinkle (*Catharanthus roseus* [L] G.Don), mango parasite (*Dendropthoe petandra* L.), white saffron (*Curcuma mangga* Val), betel leaves (*Piper betle* L.) have been reported to exhibit antioxidant, antimutation and cytotoxic that suggested the chemopreventive potential against various cancer including breast cancer. This research was conducted to investigate cytotoxic activity on breast cancer cell line T47D, antioxidant activity of *C. roseus*, *D. petandra*, *C. mangga* and *P. betle* water extracts. The cytotoxic potency was determined with MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. The antioxidant activities were determined by using *in vitro* assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. *C. roseus* water extract was able to inhibit T47D cell proliferation with IC_{50} 4%, *D. petandra* with IC_{50} 1%, *C. mangga* with IC_{50} 14% and *P. betle* with IC_{50} 3%. The highest DPPH scavenging activity of *C. roseus* was 71.87%, *D. petandra* was 75.11%, *C. mangga* was 38.45% and *P. betle* water extract was 83%. We suggest that *D. petandra*, *P. betle* and *C. roseus* water extract have a potential cytotoxic and antioxidant activities compared with *C. mangga* water extract.

Keywords : cytotoxic, antioxidant, *Catharanthus roseus*, *Dendropthoe petandra*, *Curcuma mangga*, *Piper betle*, water extract, breast cancer, T47D

Introduction

The three most commonly diagnosed types of cancer among women in 2010 were cancers of the breast, lung, and colorectum, accounting for 52% of cancer cases in this group. Breast cancer alone accounted for 28% (207,090) of all new cancer cases among women (Kaghani *et al*., 2011). Breast cancer (BC) is one of the most important causes of morbidity and mortality representing the first tumor in the female sex in terms of incidence and the third in terms of mortality in the western world (Andreetta *et al*., 2010).

The chemotherapeutic drugs including etoposide, camptothecin, vincristine, cis-platinum, cyclophosphamide, paclitaxel (Taxol), 5- fluorouracil and doxorubicin have been observed to induce apoptosis in cancer cells (Kaufman *et al*., 2000; Johnstone *et al*., 2002; Abdolmohammadi *et al*., 2008). Lipid peroxidation is a free radical mediated phenomenon in biological tissues where poly unsaturated fatty acids are generally abundant and is used parameters for assessing the involvement of free radicals in cell damage (Sinha *et al*., 2009), as evidenced by the formation of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH) and conjugated dienes (CD) as well as the status of the antioxidants

superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in breast cancer tissues was enhanced compared to control (Kumaraguruparan *et al*., 2002). Antioxidant CAT, SOD also act as anti-carcinogens and inhibitors at initiation and promotion/transformation stage in carcinogenesis. Mutation caused by potassium superoxide in mammalian cells is blocked by SOD. Plasma DNA strand scission caused by xanthine/xanthine oxidase is prevented by SOD and CAT enzymes (Sinha *et al*., 2009). The leaves extract of *P. betle* is reported to exhibit biological capabilities of detoxication, antioxidation and antimutagenic activities that suggested the chemopreventive potential of the extract against various ailments including liver fibrosis (Shun *et al*., 2007; Fatahilah *et al*., 2010). Ethanolic extarct of *P. betle* leaves is promising source as natural antioxidant and antiproliferative in breast cancer T47D cell line (Widowati et al., 2011).

Herbal medicines are usually very easily accepted by women, as many as 80% of women with breast cancer use some form of complementary or alternative medicine, the most common using herbs, lessen the side effects of treatment, improve quality of life, provide a greater sense of control, and reduce stress (Roberts, 2010; Kaghani *et al*., 2011). *C. roseus* was used as a remedy in cancer related diseases. Aerial part of the plant contains about 90 different alkaloids. Crude extract of *C. roseus* using 50 and 100% methanol had significant anticancer activity against different cell types in vitro at <15 µg/mL) (Ueda *et al*., 2002). *D. petandra* is traditionally used as cancer medicine. Its flavonoids content can inhibit growth of *Artemia salina* Leach as anticancer activity assay *in vivo* (Sukardiman *et al*., 1999). White saffron rhizome is a spice commonly used in traditional medicine. Compounds from *C. mangga* showed high cytotoxic activity against a panel of human tumor cell lines, such as human leukemia (HL-60), breast cancer (MCF-7) and liver cancer (HepG2) (Abas *et al*., 2005). Water extract of *C. mangga* exhibit antioxidant activity (Pujimulyani *et al*., 2004).

Materials and Methods

Plants materials

Materials were aerialas and roots of *C. roseus* [L] G.Don., small branches of *D. petandra* L. and rhizomes of *C. mangga* Val., levaes of *P. betle* were collected from from plantation located in Bogor, West Java, Indonesia (May, 2009). The plants were identified by staff of herbarium, department of biology, school of life sciences and technology, Bandung institute of technology, Bandung, west Java, Indonesia. The aerials and roots, leaves and branches, leaves and rhizomes were collected, chopped finely and kept under drier tunnel ($40\text{-}45^0$ C).

Preparation of extract

Ten gram of dried and chopped materials were boiled with 100 ml distilled water (aquadest) with $75\text{-}90^0$C until the remained water was 50 ml, and filtrated. The water extracts were stored at 4 ^{o}C. The water extracts of *C. roseus, D. petandra, P. betle* and *C. mangga* were dissolved in 10% dimethy sulfoxide (DMSO-Merck) and subsequently diluted to appropriate working concentrations with Dulbecco's Modified Eagle's Medium (DMEM-Sigma Aldrich) culture for proliferation inhibitor proliferative (Tan *et al.,* 2005).

Cell culture

The human breast cancer T47D cell line was obtained from the Indonesian institute of sciences, research centre for chemistry, division of natural products, food and pharmaceuticals, Bandung, West Java, Indonesia. The cells were grown and maintained in DMEM supplemented with 10% (v/v) foetal bovine serum (FBS-Sigma Aldrich), 100

units/ml penicillin (Sigma Aldrich) and 100 µg/ml streptomycin (Sigma Aldrich), and incubated at 37^0 C in a humidified atmosphere and 5% CO_2 (Mooney *et al.,* 2002; Tan *et al*., 2005).

DPPH scavenging activity assay

The DPPH assay was carried out as described by Unlu *et al* (2003). Pipette 50 µl of ethanol extracts of *C roseues, D petandra, P. betle, C. mangga*. To obtain the IC_{50} value, a range of various final concentrations was used e.g. 100, 50, 25, 12.5; 6.25, 3.125, 1.563, 0.781, 0.391 and 0.195 µg/ml introduced at the microplate and then were added 200 µL of 0.077 mmol/l DPPH (Sigma Aldrich) in methanol and the reaction mixture was shaken vigorously and kept in the dark for 30 min at room temperature, furthermore DPPH scavenging activity was determined by microplate reader at 517 nm.

The radical scavenging activity of each sample was expressed by the ratio of lowering of the absorption of DPPH (%), relative to the absorption (100%) of DPPH solution in the absence of test sample (negative control).

$$\text{scavenging \%} = \frac{A_c - A_s}{A_c} \times 100$$

As: absorbance of samples, Ac: negative control absorbance (without sample)

IC_{50} determination

The IC_{50} (median inhibition concentration) is the concentration of toxic extract that reduces the biological activity by 50 %. The IC_{50} value for cytotoxicity was obtained from the MTS assay and calculated using linear regression analysis in Microsoft Excel software. Optical density (OD) at 515 nm of cells number without treatment was established as standard curve function. Read OD of sample was converted to number of cells using standard curve equation, linear graphic of % living cells in function of extract concentrations was traced. The IC_{50} value was the concentration of toxic extracts reduced the biological activity by 50 %.

Results

Cytotoxic activityof C. roseus, D. petandra, P. betle* and *C. mangga extracts

Figure 1. shows the cell viability of T47D cells treated by P. *C. roseus, D. petandra, P. betle* and *C. mangga* extracts, the *C. roseus, D. petandra, P. betle* extracts exhibited a decrease in viability in a concentration dependent-manner. Higher concentration extracts will increase the cytotoxicity. The IC_{50} of *C. roseus, D. petandra,P. betle* and *C. mangga* extracts in T47D cells respectively were 1% ; 4%; 3% and 14% concentrations.

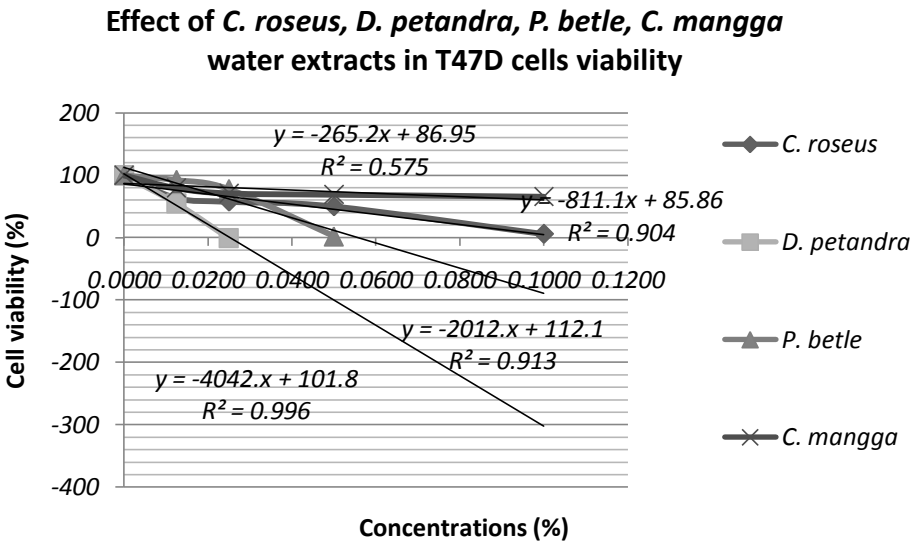

Figure 1. *C. roseus, D. petandra, P. betle* and *C. mangga* water extracts in T47D cells viability

Antioxidant activity of C. roseus, D. petandra, P. betle* and *C. mangga extracts

The DPPH free radical scavenging activity of *C. roseus, D. petandra, P betle* and *C. mangga* water extracts of various concentration were measured to examine the antioxidant activity. The IC_{50} is the concentration of antioxidants activity to scavenge DPPH free radical 50 %. Figure 2. shows the DPPH scavenging activity of *C. mangga* extract showed the lowest activity compared to *C. roseus, D. petandra, P. betle* water extract. The highest of *P. betle, C. roseus, D. petandra and C. mangga* water extracts towards DPPH scavenging activity can be seen at Table 1.

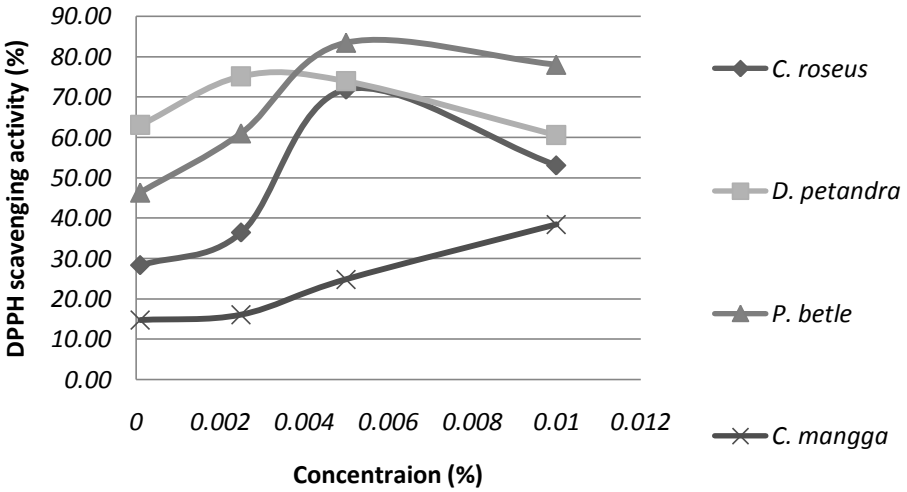

Figure 2. The DPPH scavenging activity of *C. roseus, D. petandra, P. betle* and *C. mangga* water extracts

Table 1. The highest DPPH scavenging activity

Samples	The highest DPPH scavenging activity (%)	Concentration (%)
C. roseus	71.87	0.5% (0.005)
D. petandra	75.11	0.25% (0.0025)
P. betle	83.46	0.5% (0.005)
C. mangga	38.46	1% (0.01)

Discussion

Base on the data (Figure 1.) showed that *C. roseus*, *D. petandra* and *P. betle* water extracts had cytotoxic activity with IC_{50} 4%, 1% and3%. This results were validated with previous study by Widowati *et al*. (2010a) and Widowati *et al*. (2011) that *C. roseus* ethanolic extract has cytotoxic activity, can induce apoptosis in T47D cell line. This results are consistent with previous studies the *C. roseus* extract is able to induce DNA fragmentation by gel electrophoresis. In each case, DNA fragmentation was characterised by oligonucleosomal size fragments of about 180-200 base pairs (bp), a well-known feature indicative of programmed cell deat (Compton 1992; Ahmad *et al*., 2010). Crude extract of *C. roseus* using 50 and 100% methanol had significant anticancer activity against different cell types in vitro at <15µg/mL) (Ueda *et al*., 2002). Crude decoction (200 mg and 1 g herb/mL water) showed moderate *in vitro* antiangiogenesis effects (Ghosh and Gupta, 1980; Chattopadhyay *et al*, 1991, 1992). *D. petandra* water extract showed cytotoxic activity. This results are validated with previous research that *D. petandra* is traditionally used as cancer medicine. Its flavonoids content can inhibit growth of *Artemia salina* Leach as anticancer activity assay *in vivo* (Sukardiman *et al*., 1999), but this results was not validated with previous study that water and ethanolic extracts of *D. petandra* leaves has not cytotoxic activity in melanoma cancer B16 cell line (Artanti *et al*., 2006), ethanolic extract of *D. petandra* has no cytotoxic activity in breast cancer T47D cell line with IC_{50} 728.05 µg/ml (Widowati *et al*., 2011). *P. betle* water extract showed cytotoxic activity. This results were validated with previous study by Widowati *et al*. (2011) that *P. betle* ethanolic extract has cytotoxic activity with IC_{50} 55.2 µg/ml. This results are consistent with previous studies that *P. betle* aqueos extract has antiproliferative activity towards nasopharyngeal epidermoid carcinoma cells (Fatahilah *et al*., 2010). Cytotoxic effect of *P. betle* aqueous extract on KB cells, exhibit strength antiproliferative activity towards KB cells with IC_{50} 29,5 µg/mL and do not show any cytotoxic activity even at 100 µg/ml on HeLa cells. Biologically active in the *P. betle* extract is identified as chlorogenic acid and kills myeloid and lymphoid cancer cells but normal cells are unaffected (IICB Report, 2004). The chlorogenic acid is shown to induce program cell death in human cancer cells transplanted in experimental nude mice and at the same time, shows no effect on the growth of non-cancerous cells. Those previous studies showed that *P. betle* extract has great potential to be developed as a target-specific, therapeutic drug for blood cancer (Fatahilah *et al*., 2010). *P. betle* aqueous leaves extracts have found to exhibit stronger antiproliferative activity towards human nasopharyngeal epidermoid carcinoma (KB) cells compared to their essential oils (Manosroi *et al*., 2006; Fatahilah *et al*., 2010). *C. mangga* water extract exhibited no anticancer activity in T47D cell line, with IC_{50} resulted 14%, this result was validated with previous research that ethanolic extract of *C. mangga* has no anticancer activity in T47D cell line with IC_{50} 404.76 µg/ml (Widowati *et al*., 2011).

Base on data Table 1. and Figure 2. showed that *C. roseus* water extract had antioxidant activity to scavenge DPPH free radical at level concentration 0.5% resulted71.87%. This results were not validated with previous studies that ethanolic extract of

C. roseus has no antioxidant activity (Widowati *et al*., 2010a; Widowati *et al*., 2011). *D. petandra* water extract had antioxidant activity to scavenge DPPH free radical at level concentration 0.25% resulted 75.11%. This results were validated with previous research that *D. petandra* ethanolic extract exhibit highest antioxidant activity is comparable with ascorbic acid and quercetin (Widowati *et al*., 2011). The crude decoction of *D. petandra* has high antioxidant activity (Maria, 1996), Water and ethanol extract of *D. petandra* exhibit DPPH free radical scavenging activity with IC50 < 50 µg/ml (Fajriah *et al*., 2006). Quercetin is one of the compound in *D. petandra* has high antioxidant activity (Dewiyanus, 1996; Gordon, 2001). *P.betle* water extract had antioxidant activity to scavenge DPPH free radical at level concentration 0.5% resulted 83.46%. This results were validated with previous research that *P. betle* ethanolic extract exhibit high antioxidant activity with IC_{50} 3.48 µg/ml (Risdian *et al*., 2010) and IC50 of ethanolic extract of *P. betle* is 5.49 µg/ml (Widowati *et al*., 2011). *P. betle* ethanolic extract is higher DPPH scavenging activity than *C. roseus* and C. mangga extract . Therefore, we assume that DPPH free radical scavenging activity is related to the presence of bioactive compounds such as phenolic compounds in extract. Our previous work showed that phenolic contents using kaempferol as standard, *P. betle* ethanolic extract contains high polyphenol 548.667 µg KE/mg (Widowati *et al*., 2010b), using *Epigallo Catehin Gallate* (EGCG) as standard, *P. betle* ethanolic extract contains high polyphenol 269.97µg EGCGE/mg (Risdian *et al.*, 2010). Polyphenol-rich extracts are potent DPPH scavengers offering overall protection against various stresses. P. *betle* extract shows activity similar to quercetin and protects LDL from oxidation in a dose dependent manner at concentrations higher than 10 µg/ml (Kumar *et al*., 2010). Polyphenols are one of the major plant compounds with antioxidant activity. The –OH groups in phenolic compounds are thought have a significant role in antioxidant activity (Arumugam *et al*., 2006). The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties (Rahman *et al*., 2008). Aqueous extract of *P. betle* leaves is also shown to be a scavenger of H_2O_2, superoxide radical and hydroxyl (*OH) radical (Kumar *et al*., 2010). *C mangga* water extract had no antioxidant activity to scavenge DPPH free radical at level concentration 1% resulted 38.46%, this research is very contradictory with previous research by Ruangsang *et al.* (2009) which *C. mangga* rhizomes have antioxidant, anticancer and anti-inflammatory activities. Water extract of white saffron (*C. mangga*) exhibit antioxidant activity using ß-carotene bleaching and DPPH scavenging method. Higher concentration of white saffron extract will increase the antioxidant activity, it may be due the curcuminoid content (Pujimulyani *et al*., 2004). Curcuminoid is one of the compounds in Curcuma exhibit antioxidant activity as free radical scavenger (Majeed *et al*., 1995; Pujimulyani *et al*., 2004). The antioxidative activity of curcuminoid compounds (curcumin, demethoxy curcumin and bisdemethoxy curcumin) is 20, 9 and 8 times higher compared with a-tocopherol using modified active oxygen method (Toda *et al*., 1985; Pujimulyani *et al*., 2004).

Conclusions

Water extracts of *C. roseus, D. petandra, P. betle* have cytotoxic activity in breast cancer T47 D cell line and have antioxidant activity to scavenge DPPH free radical activity. Water extract of *C. mangga* has no cytotoxic activity and antioxidant activity.

Acknowledgment

We gratefully acknowledge for the financial support of Directorate General for Higher Education, National Ministry of Republic Indonesia for research grant of Hibah Bersaing 2009-2010.

References

Abdolmohammadi, M.H., Fouladdel, S., Shafiee, A. ,Amin, G. ,Ghaffari, S.M., and Azizi, E. 2008. Anticancer effects and cell cycle analysis on human breast cancer T47D cells treated with extracts of *Astrodaucus persicus* (Boiss.) drude in comparison to doxorubicin. *DARU*., 16(2):112-118.

Ahmad, N. Rahim, R.R. and Mat, I. 2010. *Catharanthus roseus* aqueous extract is cytotoxic to Jurkat leukaemic T-cells but induces the proliferation of normal peripheral blood mononuclear cells. Tropical Life Sciences Research, 21(2), 105–115.

Andreetta, C., Minisini, A. M., Miscoria, M. and Puglisi, F. 2010. First-line Chemotherapy with or without Biologic Agents for Metastatic Breast Cancer. *Oncology Hematology*., 76(2):99-111.

Artanti, N., Oktavieni, F., Darmawan, A., Fajriah, S., Sundowo, A., Hanafi, M., Arung, E.T., Shimizu, K. and Kondo, R. 2009. Antioxidant and Cytotoxic Activities of Water and Ethanol Leaves Extracts of Saraca indica and Its Mistle. Proceeding The Henk Timmerman International Seminar & Award on Structure Based Drug Design, Yogyakarta, October 7, 2009.

Arumugam, P., Ramamurthy, P., Santhiya, S.T. and Ramesh, A. 2006. Antioxidant activity measured in different solvent fractions obtained from Mentha spicata Linn.: An analysis by ABTS.+ decolorization assay. Asia Pac. J. Clin. Nutr. 119-124.

Chattopadhyay, R.R. Banerjee, R.N., Sarkar, S.K., Ganguly, S. and Basu, T.K. 1992. Anti-inflammatory and acute toxicity studies with leaves of *Vincarosea*Linn in experimental animals. *Indian J. Physiol. Pharmacoll*., 36:291-292.

Compton, M..M. 1992. A biochemical hallmark of apoptosis: Internucleosomal degradation of the genome. *Cancer Met. Rev*., 11:105-119.

Dewiyanus, I.D. 1996. Pemeriksaaankandungan kimia benalu teh (Dendrophtoe pentandra L.) Miq. dan uji toksisitasnya dengan metoda Braine Shrimp, Abstrak no 268, Buku 10, Penelitian Tanaman Obat di Beberapa Perguruan Tinggi di Indonesia, Jakarta.

Fajriah, S., Darmawan, A., Sundowo, A. and Artatnti , N. 2006. Isolasi senyawa antioksidan dari ekstrak etil asetat daun benalu *Dendrophthoe pentandra* L. Miq yang tumbuh pada inang Lobi-Lobi. *Jurnal Kimia Indonesia*, 1(1):1-4.

Ferguson,P.J. Kuowska, E. Freeman, D.J. Chambers, A.F. and Koropatnick, D.J. 2004. A Flavonoid Fraction from Cranberry Extract Inhibits Proliferation of Human Tumor Cell Lines. *J. Nutr.* 134: 1529–1535.

Fathilah, A.R., Sujata, R., Norhanom, A.W. and Adenan, M.I. 2010. Antiproliferative activity of aqueous extract of *Piper betle* L. and *Psidium guajava* L. on KB and HeLa cell lines. *J. Med. Plant. Res*. 4(11):987-990.

Ghosh, R.K. and Gupta, I. 1980. Effect of Vincarosea and Ficusracemososus on hyperglycemia in rats. *Indian J. Anim. Health*.,19: 145-148.

IICB Report. 2004. First herb-based Cancer cure, Council for Scientific and Industrial Research's premier lab Indian Institute of Chemical Biology August 2004, http://nation.ittefaq.com/artman/publish/article_11132.shtml

Jemal, A., Siegel, R., Xu, J. and Ward, E. 2010. Cancer Statistics, 2010," *A Cancer Journal for Clinicians.,* 60(5): 277-300.

Johnstone, R.W., Ruefli , A.A., and Lowe, S.W. 2002. Apoptosis: a link between cancer genetics and Chemotherapy. *Cell*. ,108(25):153-164.

Kaufmann, S.H. and Earnshaw, W.C. 2000. Induction of apoptosis by cancer chemotherapy. *Exp. Cell Res*. 256: 42-49

Khaghani, S., Razi, F., Yajloo, M.M., Paknejad, M., Shariftabrizi, A. and Pasalar, P. 2011. Selective Cytotoxicity and Apoptogenic Activity of Hibiscus Sabdariffa Aqueous Extract against MCF-7 Human Breast Cancer Cell Line. *J. Cancer Therapy*., 2:394-400

Kumaraguruparan, R., Subapriya, R., Viswanathan, P., Nagini, S. 2002. Tissue lipid peroxidation and antioxidant status in patients with adenocarcinoma of the breast. *Clinica Chimica Acta*. 325(1-2):165-170.

Kumar, N., Misra, P., Dube, A., Bhattacharya, S., Dikshit, M., Ranade, S.2010. *Piper betle* Linn. a maligned Pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery. Current Sci. 99(7):922-932.

Majeed, M., Vladimir ,B., Uma, S., Rajendran,R. 1995. Curcuminoids Antioxidant Phytonutriens. Nutriscience. New Jersey.

Malich, G. Markovic, B. and Winder, C.1997. The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. *Toxicol*.,124(3):179-189.

Manosroi, J., Dhumtanom, P., Manosroi, A.2006. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Lett. 235: 114-120

Maria, N. 1996. *Dendrophtoe petandra* (L) Miq. Analisis daya antioksidan beberapa jenis benalu secara spektrofotometri dan potensiometri. Abstrak no 270, Fakultas MIPA. Buku 10, Penelitian Tanaman Obat di Beberapa Perguruan Tinggi di Indonesia, Jakarta.

Mooney, L.M. Al-Sakkaf, K.A. Brown, B.L. and Dobson, P.R.M. 2002. Apoptotic mechanisms in T47D and MCF-7 human breast cancer cells. *British J. Cancer*., 87:909–917.

Pujimulyani, D., Wazyka, A., Anggrahini, S. and Santoso, U. 2004. Antioxidative properties of white saffron extract (*Curcuma mangga* Val) in the ß-carotene bleaching and DPPH-radical scavening methods. Indonesian Food and Nutr. Progress. II(2): 35-40.

Rahman, K. 2007. Studies on free radicals, antioxidants, and cofactors. *Clin Interv Aging*. 2(2): 219–236.

Risdian, C. Widowati, W. Mozef, T. Wargasetia, T.L. Khiong, K. 2010. *In vitro* free radical scavenging activity of ethanolic leaves extract and its different solvent fractions of *Piper betle* L., *Ind. J Cancer Chemoprevention*, 2(1), 7-11.

Roberts, H. 2010. Safety of Herbal Medicinal Products in Women with Breast Cancer. *Maturitas,* 66(4):363-369.

Ruangsang, P., Tewtrakul, S., Reanmongkol, W. 2009. Evaluation of analgesic and anti-inflammatory of *Curcuma mangga* Val and Zijp rhizomes. *J. Nat. Med.*DOI.10.1007/sII1418:0365-1

Sukardiman, Santa, I.G.P. and Rahmadany. 1999. Efek Antikanker Isolat Flavonoid dari Herba Benalu Mangga (Dendrophtoe petandra). Cermin Dunia Kedokteran. 122: 5-8.

Tan, M.L. Sulaiman, S.F. Najimuddin, N. Samian ,M.R. and Muhammad, T.S.T. 2005,.Methanolic extract of *Pereskia bloe* (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47D cell line. *J. Ethnopharmacol*. 96:287-294.

Toda, S., Miyase, T. Archi, H., Takino, Y. 1985. Natural antioxidant III, antioxidative components, isolated from rhizoma of *Curcuma longa* L. Chem. Pharm. Bull. 33:1725-1728.

Ueda, J.Y. Tezuka, Y. Banskota , A.H., Le Tran, Q. and Tran, Q.K. 2002 Antiproliferative activity of Vietnamese medicinal plants. *Biol. Pharm. Bull*., 25: 753-760.

Unlu, G.V., Candan, F., Sokmen, A., Dafefera, D., Polissiou, M., Sokmen, E., Donmez, and Tepe ,B. 2003. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of Thymus pectinatus Fisch. Et Mey. Var. pectinatus (Lamiaceae). *J. Agric. Food Chem.* 51:63-67.

Sinha R.J., Singh, R., Mehrotra, S. and Singh, R.K. 2009. Implications of free radicals and antioxidant levels in carcinoma of the breast: A neverending battle for survival. *Indian J. Cancer*. 46(2):146-150.

Shun, C.Y., Chau, J.W., Jing J.L., Pei, L.P., Jui, L.H. and Fen, P.C. 2007. Protection effect of *Piper betle* leaf extract against carbon tetrachloride-induced liver fibrosis in rats. *Arch. Toxicol.* 81: 45-55.

Widowati, W., Mozef, T., Risdian, C., Ratnawati, H., Tjahyani, S., Sandra, F. 2010a**.** Apoptosis and Antioxidant Activities of Catharanthus roseus [L] G. Don Extrcat on Breast Cancer Cell Line. *Indonesian Journal of Cancer Chemoprevention*. 1(2): 99-107.

Widowati, W., Mozef, T., Risdian, C., Wargasetia, T.L., Khiong K. and Sandra, F. 2010b. Antioxidant and apoptosis activities of Piper betle Linn. Leaves extract on HeLa cervical cancer cell Line. *Simposium Nasional Kimia Bahan Alam* XVIII. Bandung 9-10 November 2010

Widowati, W. Tjandrawati, M. Risdian, C. Ratnawati, H. Tjahjani, S. and Sandra, F, 2011, The Comparison of Antioxidative and Proliferation Inhibitor Properties of *Piper betle* L., *Catharanthus roseus* [L] G.Don, *Dendrophtoe petandra* L., *Curcuma mangga* Val. Extracts on T47D Cancer Cell Line. *Int. Res. J. Biochem Bioinfor* m 1(12): 022-028.

Yang, C. S. Landau, J. M. Huang, M. T. and Newmark, H. L., 2001, Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu. Rev. Nutr*. 21: 381–406.

.