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Full Length Research Paper

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

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Breast cancer is the most common cancer among women. It is estimated that one of eight women will be diagnosed with breast cancer in women. The betel leaves (*Piper betle* L.), madagascar prewinkle (*Catharanthus roseus* [L] G.Don), mango parasite (*Dendropthoe petandra L.*) and white saffron (*Curcuma mangga* Val.) have been reported to exhibit antioxidant, and antimutation that suggested the chemopreventive potential against various cancer including breast cancer. This research was conducted to investigate anticancer activity of *P. betle, C. roseus, D. petandra* and *C. mangga* extracts on breast cancer cell line T47D, and antioxidant activity. The anticancer activity was determined with MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. The antioxidant activity was determined by using *in vitro* assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. *P. betle* , *C. roseus* extracts were able to inhibit T47D cell proliferation with IC_{50} 55.2 µg/ml, 26.22 µg/ml and *D. petandra*, *C. mangga* extracts with IC_{50} 728.05 µg/ml, 404.76 µg/ml, while DPPH scavenging activity (IC_{50}) on *P. betle, C. roseus, D. petandra, C. mangga* extracts were respectively 5.49 µg/ml, 102.96 µg/ml, 4.74 µg/ml, 277.79 µg/ml. *P. betle* and *D. petandra* extracts are more active antioxidant compared to *C. roseus* and *C. mangga*.

Key words : *Piper betle* L., *Catharanthus roseus* [L] G.Don, *Dendropthoe petandra L., Curcuma mangga* Val., antioxidant, anticancer, DPPH

INTRODUCTION

Breast cancer is the most common cancer among women and the second leading cause of cancer deaths in women after lung cancer (Lopez and Sekharam, 2008). It is estimated that one of eight women will be diagnosed with breast cancer in women (Chen and Yan, 2007). Cancer chemoprevention applies specific natural or synthetic chemical compounds to inhibit or reverse carcinogenesis and to suppress the development of cancer from premalignant lesions (Sarkar and Li, 2007; Abdolmohammadi *et al*., 2009). A major problem with present cancer chemotherapy is the serious deficiency of active drugs for the curative therapy of tumors (Valeriote *et al*., 2002; Kinghorn *et al*., 2003; Abdolmohammadi *et al*., 2009). The chemotherapeutic drugs including etoposide, camptothecin, vincristine, cis-platinum, cyclophosphamide, paclitaxel (Taxol), 5- fluorouracil and

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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 one of the most frequently used parameters for assessing the involvement of free radicals in cell damage. The probable reason for the elevated level of serum lipid peroxide in breast carcinoma may be due to defective antioxidant system which leads to the accumulation of lipid peroxides in cancer tissue which are released into the blood stream. In breast cancer tissue, the malondialdehyde (MDA) level in stage IV was significantly higher as compared to stage I indicating increased free radical activity with increasing severity of cancer (Sinha *et al*., 2009). Lipid peroxidation 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).

One of the approaches used in drug discovery, is the ethnomedical data approach, in which the selection of a plant is based on the prior information. An ethanolic leaves extract of *Piper betle* has anti-inflammatory activity (Ganguly *et al*., 2007). 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). *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 anti-cancer activity against different cell types *in vitro* at <15 µg/mL) (Ueda *et al.,*2002). Crude decoction (200 mg and 1 g herb/mL water) showed moderate *in vitro* anti-angiogenesis effects (Ghosh and Gupta, 1980; Chattopadhyay *et al*, 1991, 1992). *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).

The objective of this research was to examine proliferative inhibitor property and the antioxidant activity of the *P. betle* leaves, aerials and roots of *C. roseus*, leaves and small branches of *D. petandra* and *C. mangga* rhizomes extracts.

MATERIALS AND METHODS

Plant material

Leaves of *P. betle* L., aerials and roots of *C. roseus* [L] G.Don., leaves and small branches of *D. petandra* L. and rhizomes of *C. mangga* Val. 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 fresh leaves, aerials and roots, leaves and branches and rhizomes were collected, chopped finely and kept under dry tunnel (40-45^{0}C).

Preparation of extract

One kilogram of dried and chopped materials were extracted with distilled ethanol by maceration method for 5 days, filtered then evaporated using rotatory evaporator to produce ethanol extract of *P. betle* 16.513 g/100 g , *C. roseus* 16.25 g/100 g, *D. petandra* 14.522 g/100 g and *C. mangga* 6.744 g/100 g of dried materials. The ethanol extracts were stored at 4 ^{o}C. The *P. betle, C. roseus, D. petandra* and *C. mangga* extracts were dissolved in dimethyl sulfoxide (DMSO-Merck, South St Paul) and subsequently diluted to appropriate working concentrations with Dulbecco's Modified Eagle's Medium (DMEM-Sigma Aldrich, St. Louis, MO, USA) culture for proliferation inhibitor proliferative (Tan *et al*., 2005) while four extracts *P betle; C. roseus, D. petandra* and *C. mangga* extracts were dissolved in methanol HPLC grade (Merck) for antioxidant assay.

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).

Cell viability assay

To determine cell viability, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)

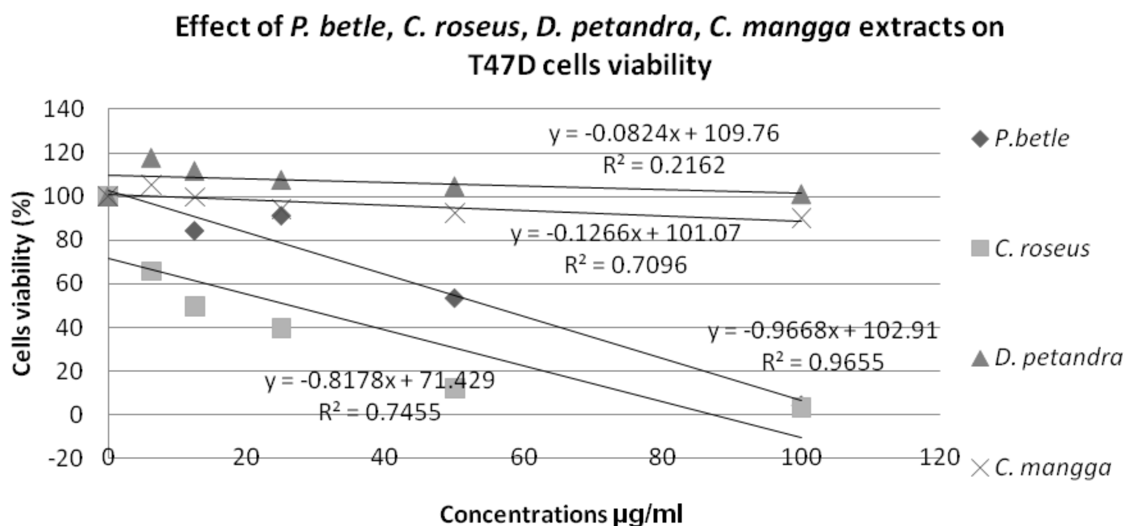

Figure 1. Effect *P. betle, C. roseus, D. petandra* and *C. mangga* extracts on T47D cells viability

(Promega, Madison, WI, USA) assay was performed according to the method described by Malich *et al.* (1997). Cell viability assay used an optimized reagent containing resazurin converted to fluorescent resorufin by viable cells that absorbes the light at 490 nm. Briefly, the cells were seeded into a 96-well plate ($5x10^4$ cells per well). After 24 h incubation, cells were supplemented by *P. betle*, *C. roseus, D petandra*, *C. mangga* extracts with various concentrations, then incubated for 24 h. Cells non supplemented with extract were used as negative control. MTS was added to each well at a ratio 1:5. The plate was incubated at 5% CO_2, 37° C for 2-4 h. The absorbance of cells was measured at 515 nm with a microplate reader. The data were presented as percent of viable cells (%) and analyzed by calculating the median Inhibition Concentration (IC_{50})

DPPH scavenging activity assay

The DPPH assay was carried out as described by Unlu *et al* (2003). Pipette 50 µl of ethanol extracts of *P. betle, C roseues, D petandra, C. mangga*, ascorbic acid (Sigma Aldrich) and quercetin (Sigma Aldrich). 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.

IC_{50} determination

The IC_{50} (median inhibition concentration) is the concentration of toxic extract that reduces the biological activity by 50 % and reduces 50% the DPPH free radical. 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 %.

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).

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

A_s: absorbance of samples, A_c: negative control absorbance (without sample)
The IC_{50} value for antioxidant activity is the value of extract concentration where there is 50 % of DPPH scavenging calcutaled using linear regression analysis.

RESULTS

Proliferative inhibitor of *P. betle*, *C. roseus, D. petandra* and *C. mangga* extracts on T47D cells

Figure 1. shows the cell viability of T47D cells treated by *P. betle*, *C. roseus, D. petandra* and *C. mangga* extracts, the extracts exhibited a decrease in viability in a concentration dependent-manner. Higher concentration

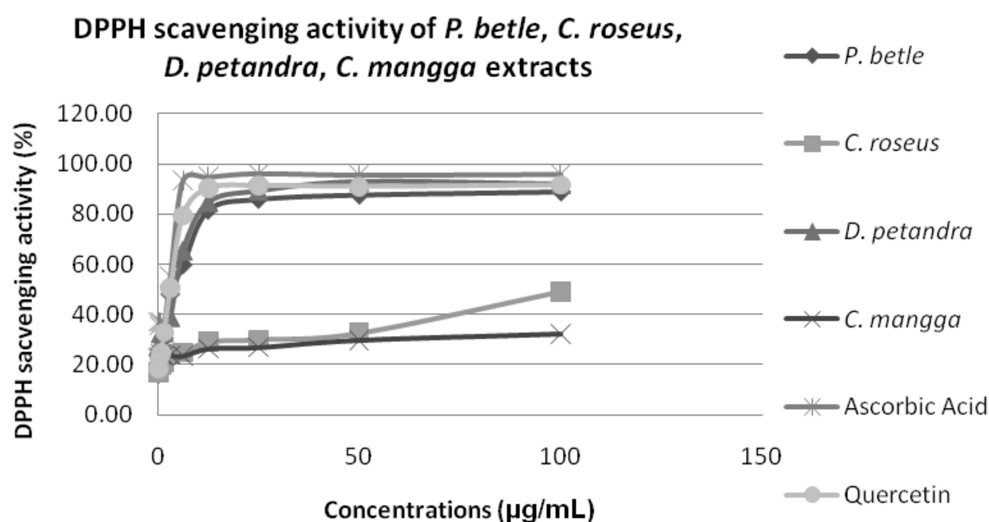

Figure 2. The DPPH scavenging activity of *P. betle, C. roseus, D.petandra* and *C. mangga* extracts

of *P. betle*, *C. roseus, D. petandra* and *C. mangga* extracts will increase the cytotoxicity. The IC_{50} of *P. betle C. roseus, D. petandra* and *C. mangga* extracts in T47D cells respectively were 55.2 µg/ml, 26.22 µg/ml, 728.05 µg/ml, 404.76 µg/ml.

DPPH scavenging activity

The DPPH free radical scavenging activity of *P betle, C. roseus, D. petandra* and *C. mangga* ethanol extracts, ascorbic acid and quercetin well known as positive control 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 *D. petandra, P. betle, C. roseus,* extract, ascorbic acid and quercetin. The IC_{50} of *P. betle, C. roseus, D. petandra* and *C. mangga* extracts towards DPPH scavenging activity can be seen at Table 1.

DISCUSSION

Base on the data (Figure 1.) showed that *C. roseus* and *P. betle* ethanol extracts had cytotoxic activity with IC_{50} 26.22 µg/ml and 55.2 µg/ml, but less active compared to doxorubicin with IC_{50} 1.74 µg/ml (our unpublished previous research). This result was validated by previous research that the antiproliferative activity of *C. roseus* caused by the most abundant ones are the monomers like catharanthine and vincloline. Two of the common anti-cancer drugs from *C. roseus* are vincristine and vinblastine. 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). Catharanthin, similar to the compounds in the plasma of cancer cells. Absorption of catharanthin into cancer cells is forecasted to be urgent and dissolve the nucleus of cancer cells. Crude decoction (200 mg and 1 g herb/mL water) showed moderate *in vitro* anti-angiogenesis effects (Ghosh and Gupta, 1980; Chattopadhyay *et al*, 1991, 1992).

P. betle had proliferative inhibitor activity, this result was validated by previous research 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

Table 1. The DPPH scavenging activity (%)

Sample	IC_{50} (µg/ml)
P. betle extract	5.49
C. roseus extract	102.96
D. petandra extract	4.74
C. mangga extract	277.79
Ascorbic acid	2.16
Quercetin	3.244

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).

On the other hand the data represented in Figure 1. showed that *D.petandra* ethanol extract was very low antiproliferative activity or had no anticancer activity (IC_{50} = 728.05 µg/mL). This result was validated with previous research that crude decoction of *D. petandra* on rats are treated with dimetilaminobenzena (DAB) as carcinogenic agent shows untoxic (Windarti, 1990), but dichlorometan extract of *D. petandra* (L.) Miq has anticancer activity according Brine Shrimp Letahlity Test (BST) in *A. salina* Leach larva for 24 hours with median lethal cytotoxicity (LC_{50}) 232.4104 µg/mL (Wahjudi, 1996). Ethanol extract and flavonoid glycoside namely 5,7,3',4'-tetrahydroxy-3-0 ramnosida flavonol or quercetin from *D. petandra* (L.) Miq have no anticancer activity according to BST method with LC_{50} > 1000 µg/ml (Dewiyanus, 1996).

This result *C. mangga* ethanol extract was very low antiproliferative activity or had no anticancer activity (IC_{50} = 404.76 µg/ml). This result was not validated with previous research. Previous by Simnadari *et al*. (2004) exhibit that protein fraction isolated from fresh *C. mangga* Val. has high cytotoxic effect on HeLa cell line and Raji cell line. Mix volatile oil of *C. mangga* using concentration of 125 µg/ml produce the highest cytotoxic effect on Raji and Myeloma cell lines, *C. mangga* oil inhibit growth of cells, induce apoptosis by increasing expression of p53 (Verliana *et al*., 2005). According to previous research that *C. mangga* contain many compounds such as a curcumin which exhibit cytotoxic and antiinflammatory activity (Aggarwal *et al*., 2006; Jain *et al*., 2007).

DPPH scavenging activity of *P. betle* and *D. petandra* extracts were higher than *C. roseus* and *C. mangga* extracts but its were comparable with ascorbic acid and quercetin (Table 1).

Base on the data (Table 1) shows that *P. betle* extract was 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* L. extract contains high polyphenol 548.667 µg KE/mg (Widowati *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 radical (Kumar *et al*., 2010).

D. petandra extract exhibited highest antioxidant activity was comparable with ascorbic acid and quercetin, this result was verified with previous research, that 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 IC_{50} < 50 µg/ml (Fajriah *et al*., 2006). Quercetin is one of the compound in *D. petandra* has high antioxidant activity (Dewiyanus, 1996; Gordon, 2001).

Base on data (Table 1) shows that *C. mangga* and *C. roseus* extract had no antioxidant activity, this 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 β-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 α-tocopherol using modified active oxygen method (Toda *et al*., 1985; Pujimulyani *et al*., 2004).

CONCLUSION

Ethanol extract of *P. betle* is promising source as natural antioxidant and antiproliferative. Ethanol extract *C. roseus* has antiproliverative activity, but its does not have antioxidant activity. Ethanol extract of *D. petandra* extract has high antioxidant activity, but its does not have antiproliverative activity. Ethanol extracts of *C. mangga* does not have antioxidant and antiproliverative properties.

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