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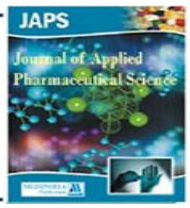

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Bioactivities Evaluation of Indonesian Mistletoes (*Dendrophthoe pentandra* (L.) Miq.) Leaves Extracts

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ABSTRACT

Mistletoes or *benalu* in bahasa Indonesia is a semi-parasitic plant that also known as medicinal plant. It used in traditional/alternative medicine such as for for cough, diabetes, hypertension, cancer, diuretic, smallpox, ulcer, skin infection and after child-birth treatment. There are many species of mistletoes in Indonesia. *Dendrophthoe pentandra* (L.) Miq. is one of the Indonesian mistletoes species that commonly found grew on many different species of host plant. In this paper we reported *in vitro* toxicity, antioxidant and antidiabetes activities of MeOH and water extracts of *D. pentandra* grew on four different host plants (*Stelechocarpus burahol*, *Spondias dulcis*, *Annona squamosa* and *Camellia sinensis*). Toxicity was measured using brine shrimp lethality test (BSLT). Antioxidant activity was measured using DPPH free radical scavenging assay. Antidiabetes activity was measured using α-glucosidase inhibitor assay. The results show that all mistletoes extracts tested (MeOH and water extracts) were non-toxic and show significant antidiabetes activity, whereas for antioxidant activity, only MeOH extracts show significant activity. Therefore, it is suggest that *D. pentandra* extracts are potential source for natural antioxidant and antidiabetes compounds.

Keywords: *Dendrophthoe pentandra* (L.) Miq., medicinal plant, BSLT, antioxidant, DPPH, antidiabetes, α-glucosidase.

INTRODUCTION

Although there are many chemical synthetic drugs, biodiversity from nature particularly plants is still the important source of medicinal products since the past century (Cragg *et al*., 2009). In the review by Newman and Cragg (2007) it was stated that in the area of cancer drugs, from 155 small drugs molecules , 47% derived from or natural products itself. Therefore, exploration of new leads for drug discovery and development from plants is still important (Hamid *et al*., 2011). For the country like Indonesia, that is rich in plant biodiversity, opportunity to find new leads for drug discovery need to be explored by investigating the bioactivities of plants that already used for traditional/alternative medicine. Mistletoes or *benalu* in Bahasa Indonesia is a semi-parasitic plants that also known as medicinal plant. As a semi-parasitic plant, mistletoe is considers as an unwanted plant to economically important horticultural plant, however in the other side, mistletoe is known as one of medicinal plant used in traditional/alternative medicine in Indonesia and other countries such as in treatment for cough, diabetes, hypertension, cancer, diuretic, smallpox, ulcer, skin infection and after child-birth (Ishizu *et al.*, 2002; Valkenburg,

Osabede *et al.*, 2004). There are many species of mistletoe. It was reported that there were 44 species of mistletoe in Java (Indonesia) which belong to the family of Loranthaceae, Santalaceae and Viscaceae. (Windari and Rahajoe, 1998). However people in Indonesia usually called the mistletoe depend on the host plant where it grew, such as *benalu teh* (mistletoe that grew on tea as host plant). This way of naming mistletoe can be misleading. Different species of mistletoes can grew on the same host tree or the other way around one species of mistletoe can grew on many different host tree. Since mistletoe is a semi-parasitic plant, it is suggests that their bioactivities could also depend on their host plant (Xiou *et al.*, 2008). *Dendrophthoe pentandra* (L.) Miq. is one of the Indonesian mistletoes species which belong to the family of Loranthaceae. This species in commonly found grew on many different species of host plant (Valkenburg 2003; Huaxing *et al*., 2003). The aim of this present study was to evaluate *in vitro* toxicity, antioxidant and antidiabetes activities of MeOH and water extracts of *D. pentandra* grew on four different host plants (*Stelechocarpus burahol*, *Spondias dulcis, Annona squamosa* and *Camellia sinensis*). Toxicity was measured using brine shrimp lethality test (BSLT) (Meyer *et al.*, 1982). This method is often used for preliminary screening before cytotoxicity assay using cancer cell line. Antioxidant activity was measured using DPPH free radical scavenging assay (Yen and Chen, 1995). Antioxidant is a compound that has ability to inhibit oxidation rate or to neutralize a free radicals. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis (Halliwell and Gutteridge, 1984). Antioxidant supplements, or foods containing antioxidants, may be used to help the human body reduce oxidative damage (Yang *et al.*, 2002). Antidiabetes activity was measured using α-glucosidase inhibitor assay (Kim *et al.*, 2004). The α-glucosidase is the enzyme that catalyzes the cleavage of glycosidic bonds in oligosaccharides. Compound that can inhibit the activity of this enzyme is considered having antidiabetes activity because it could help preventing postprandial hyperglycemia by decreasing the rate of carbohydrate degradation to glucose (Kim *et al.*, 2004).

MATERIALS AND METHODS

Plant Materials

List of mistletoes used is shown in Table 1. Fresh leaves of *D. pentandra* were collected around Serpong, Banten Province, Indonesia or purchaced from traditional market. The samples were sent to the Herbarium Bogoriense, Research Centre for Biology-Indonesian Institute of Sciences, Bogor, Indonesia for determination of their scientific name.

Extraction

Leaves of *D. pentandra* were dried in 50°C forced fan oven. Dried leaves of *D. pentandra* from various host 2 g each were extracted in 30 ml methanol (MeOH) for 3 times. The MeOH extract were dried by evaporation under vacuum. After extraction with MeOH the residue were dried in 50°C forced fan oven. The dried residues were extracted by 15 minutes boiling in 50 ml of water for 4 times. The water extracts were dried in 50°C forced fan oven until constant weight.

Table. 1: List of local name and latin name of mistletoe and its host used in this study.

S. No.	Local Name	Latin name
1	*Benalu Kepel*	*D. pentandra* grew on *Stelechocarpus burahol* (Annonaceae)
2	*Benalu Kedondong*	*D. pentandra* grew on *Spondias dulcis* (Anacardiaceae)
3	*Benalu Srikaya*	*D. pentandra* grew on *Annona squamosa* (Annonaceae)
4	*Benalu teh*	*D. pentandra* grew on *Camellia sinensis* (Theaceae)

Brine Shrimp Lethality Test (BSLT)

BSLT as general bioassay to measure *in vitro* toxicity of the samples was conducted according to the method described in Meyer *et al.* (1982). The lethality of the samples to brine shrimp (*Artemia salina*) was determined after 24 hours of exposure. Sample is consider toxic to brine shrimp if LC_{50}<1000 µg/ml

Antioxidant assay

DPPH free radical scavenging activity was conducted according to Yen and Chen (1995). Various concentrations of the mistletoe extract/fractions in 0.8 ml MeOH were mixed with 0.2 ml of Methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, resulting in a final concentration of the DPPH of 0.2 mM and sample concentrations up to 100 µg/mL. The mixture was shaken vigorously and left to stand for 30 min in room temperature, the absorbance was then measured using spectrophotometer at 515 nm. Percentage of inhibition (free radical scavenging activity) was calculated by the equation: [1 - (B/A)] x 100%; whereas A is absorbance in the absence of sample and B is absorbance in the presence of sample. IC_{50} value is denote the concentration of sample required to scavenge 50% DPPH free radicals. Samples are considered active if IC_{50}<100 µg/ml.

Alpha-glucosidase inhibitory assay

The α-glucosidase inhibitory assay was conducted according to Kim *et al*. (2004). Sample (0.1 ml) was added to a test tube containing 0.1 ml of 20 mM pNPG (*p*-Nitrophenyl □-D-glucopyranoside) and 2.2 ml of 100 mM phosphate buffer at pH 7.0, and then incubated for 5 mins at 37°C. The reaction was initiated by addition of 0.1 ml of enzyme solution (1mg/0.1ml) followed by 15 min incubation at 37°C. The reaction was stopped by addition of 2.5 ml of 200 mM Na_2CO_3. The absorbance of p-nitrophenol released from PNPG at 400 nm was measured with a spectrophotometer.

Percentage of inhibition on the α-glucosidase activity was calculated by the equation: [1 - (B/A)] x 100%; whereas A is absorbance in the absence of sample and B is absorbance in the presence of sample. IC_{50} value is denotes the concentration of sample required to inhibit 50% α-glucosidase activity. Samples are considered active if IC_{50}<100 µg/ml.

RESULTS AND DISCUSSION

BSLT

The BSLT results of both MeOH and water of *D. pentandra* leaves extract from various host shows that these extract were not toxic because all have LC_{50}>1000 µg/ml (Table 2). Hence these extracts are relatively safe to be consumes as traditional/alternative medicine. On the other hand this result could be discouraging as evidence for the used of *D. pentandra* for cancer traditional/alternative medicine, since BSLT is usually used for preliminary screening for bioactivity including for anticancer (Meyer *et al.*, 1982; Pisutthanan et al., 2004; Hamid *et al.*, 2011). Though this results is in accordance with our results of BSLT from other Indonesian mistletoe species *Macrosolen cochinchinensis* grew on jackfruit (*Artocarpus heterophyllus*) (Artanti *et al.*, 2005). In that study it was found that water and ethanol extracts of *M. cochinchinensis* leaves and stem extracts had LC_{50}>1000 µg/ml, however those extracts at concentration 100 µg/ml show cytotoxic activity by reducing B16 melanoma cell viability to 51.0-79.5% (Artanti *et al.*, 2005). Further studies using cancer cell lines should be conducted on *D. pentandra* extracts to know if they also have anticancer potential like the *M. cochinchinensis* extracts. If the extract is found to be not toxic in BSLT but found to be toxic in cancer cell line assay, it could be a good indication that the cytotoxic compounds in that extracts might have more specificity to cancer cells and hopefully less toxic to normal cells.

Table. 2: Results of toxicity analysis of various mistletoes extracts.

S. No.	Common Name	LD_{50} (µg/ml)	
		MeOH	Water
1	*Benalu Kepel*	>1000	>1000
2	*Benalu Kedondong*	>1000	>1000
3	*Benalu Srikaya*	>1000	>1000
4	*Benalu teh*	>1000	>1000

Antioxidant Activity

The results of antioxidant activity using DPPH free radical scavenger method of MeOH and water extracts of mistletoe samples is shown in Table 3. Only MeOH extracts of *D. pentandra* leaves extract from various host show significant antioxidant activity. The highest activity was from *D. pentandra* grew on *Stelechocarpus burahol (*Annonaceae) extract with IC_{50} 21.5 µg/ml This suggest that the antioxidant compound only present in MeOH extracts but not in water extracts, and different host might have affected the content of antioxidant compound in the mistletoe. Different DPPH free radical scavenging activity depending on the host also has been reported from Viscum album leaves MeOH extracts (Onay-Uçar *et al.*, 2006). Therefore *D. pentandra* is a potential source of antioxidant.

Table. 3: Results of antioxidant activities of various mistletoes extracts.

S. No.	Local Name	IC_{50} (µg/ml)	
		MeOH	Water
1	*Benalu Kepel*	21.5	299
2	*Benalu Kedondong*	30.9	445
3	*Benalu Srikaya*	22.9	741
4	*Benalu teh*	84.9	303

Antidiabetes Activity

The results of antidiabetes activity using α-glucosidase inhibition assay of MeOH and water extracts of mistletoe samples is shown in Table 4. Both MeOH and water extracts of *D. pentandra* leaves extract from various host show significant α-glucosidase inhibition activity. The highest activity was from water extract of *D. pentandra* grown on *Camellia sinensis* (Theaceae) IC_{50} 11.8 µg/ml. This suggests that the antidiabetes compound were present in MeOH and water extracts, and different host might have affected the content of antidiabetes compound in the mistletoe. This resuslts is a scientific proof of *D. pentandra* used as traditional/alternative medicine in diabetes treatment. Although not reported having α-glucosidase inhibition activity, in vivo experiment using normal and diabetic induced rats showed that other species of mistletoe such as *Viscum album* (Orhan *et al.*, 2005; Eno *et al.*, 2008) and *Loranthus micranthus* (Osabede *et al.*, 2004) also have antidiabetes activity and also affected by host plant. Therefore *D. pentandra* is also a potential source of antidiabetes compound, and further in vivo study on *D. pentandra* extracts as antiadiaetes should be conducted.

Table. 4: Results of antidiabetes activities of various mistletoes extracts.

S. No.	Common Name	IC_{50} (µg/ml)	
		MeOH	Water
1	*Benalu Kepel*	31.8	29.4
2	*Benalu Kedondong*	41.2	34.1
3	*Benalu Srikaya*	50.9	13.9
4	*Benalu teh*	17.6	11.8

CONCLUSIONS

MeOH extracts of *D. pentandra* from various hosts show significant antioxidant and antidiabetes activity, whereas the water extract only show antioxidant activity. Therefore, it is suggest that *D. pentandra* extracts are potential source for natural antioxidant and antidiabetes compounds, that different host might affected the activity. Result of this study could be the evidence of the use of this mistletoe for various treatments in traditional/alternative therapy. No toxicity on BSLT results of all extracts could be a good indication that this plant is relatively non toxic, thus it is relatively save to consume for traditional/alternative medicine. Further studies on isolation of the bioactive compounds and mechanism of how host affected the content of bioactive compounds are needed.

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REFERENCES

Artanti, N, Yudistira, I, Hanafi, M, Arung ET, Shimizu, K & Kondo R. Bioactivities of Water and Ethanol Extracts of Jackfruit (*Artocarpus heterophyllus*) Wood, Bark and Leaves and Its Mitletoe (*Macrosolen cochinchinensis*). *Proceedings of the 6th International Wood Science Symposium, LIPI-JSPS, Bali,* August 29-31, 2005, p.340-345.

Eno AE, Ofem OE, Nku CO, Ani EJ, Itam EH. Stimulation of insulin secretion by Viscum album (mistletoe) leaf extract in streptozotocin-induced diabetic rats. Afr *J Med Med Sci*. 2008; 37(2):141-147

Cragg, GM, Grothaus PG & Newman DJ. Impact of Natural Products on Developing New Anti-Cancer Agents. *Chem. Rev*. 2009, *109,* 3012–3043

Halliwell,B & Gutteridge JMC. Oxygen toxicity,oxygen radicals, transition metals and disease. *Biochemical J*., 1984; 219 ,1 –4.

Hamid, K, Sultana, S, Urmi, KF, Ullah, MO, Hasanat, A, Zulfiker & Hossain AH. *In vitro* Free Radical Scavenging and Brine Shrimp Lethality Bioassay of Aqueous Extract of *Ficus racemosa* Seed. *Jordan J. Biol. Sci.* 2011, 4(1), 51 – 54.

Huaxing, Q, Hua-hsing, C, Hua-xing, K & Gilbert, MG. Loranthaceae. *Flora of China* 2003, 5: 220-239

Ishizu, T, Winarno, H, Tsujno, E, Morita, T & Shibuya, H. Indonesian Medicinal Plants. XXIV. Stereochemical structure of Perseitol-K^+ complex isolated from the leaves of *Scurrula fusca* (Loranthaceae), *Chem. Pharm. Bull.* 2002, 50 (4): 489-492

Kim, YM, Wang, MH, & Rhee, HI. A novel □-glucosidase inhibitor from pine bark. *Carbohydrate Res*. 2004, 339: 715-717

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nicholsand DE, Mclaughlin JL. Brine shrimp; a convenient general bioassay for active plant constituents, *Planta Medica* 1982, 45: 31-34

Newman D J. and Cragg, G M. Natural Products as Sources of New Drugs over the Last 25 Years. *J. Nat. Prod.* 2007, 70: 461-477

Onay-Uçar E, Karagöz A, & Arda N. Antioxidant activity of Viscum album ssp. album. *Fitoterapia*. 2006;77(7-8):556-560.

Orhan DD, Aslan M, Sendogdu N, Ergun F & Yesilada E. Evaluation of the hypoglycemic effect and antioxidant activity of three *Viscum album* subspecies (European mistletoe) in streptozotocin-diabetic rats. *J Ethnopharmacol*. 2005;98(1-2):95-102.

Osadebe PO, Okide GB, Akabogu IC. Study on anti-diabetic activities of crude methanolic extracts of Loranthus micranthus (Linn.) sourced from five different host trees. *J Ethnopharmacol.* 2004;95(2-3):133-138.

Pisutthanan, S, Plianbangchang P, Pisutthanan N, Ruanruay S, & Muanrit O. Brine Shrimp Lethality Activity of Thai Medicinal Plants in the Family Meliaceae. *Naresuan University J.* 2004; 12(2): 13-18

Valkenburg, JLCH. (2003). *Dendrophthoe pentandra* (L.) Miq. In RHMJ Lemmens and N Bunyapraphatsara (Eds.) *Plant Resources of South East Asia (PROSEA) 12, Medicinal and Poisonous Plants 3*. (pp. 159-158). Bogor, Indonesia: Prosea Foundation.

Windari, FI & Rahajoe, JS. Biodiversity of mistletoe in Java Island. *Warta Tumbuhan Obat Indonesia*, 1998, 4, 25-29 (in Bahasa Indonesia)

Xiao YJ, Chen YZ, Chen BH, Chen JH, Lin ZX, Fan YL. Study on cytotoxic activities on human leukemia cell line HL-60 by flavonoids extracts of Scurrula parasitica from four different host trees . *Zhongguo Zhong Yao Za Zhi*. 2008;33(4):427-32.

Yang, J, Lin, H & Mau J. Antioxidant properties of several commercial mushrooms. *Food Chem.*, 2002, 77, 229-235

Yen G & Chen, H. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem*., 1995, 43: 27-32