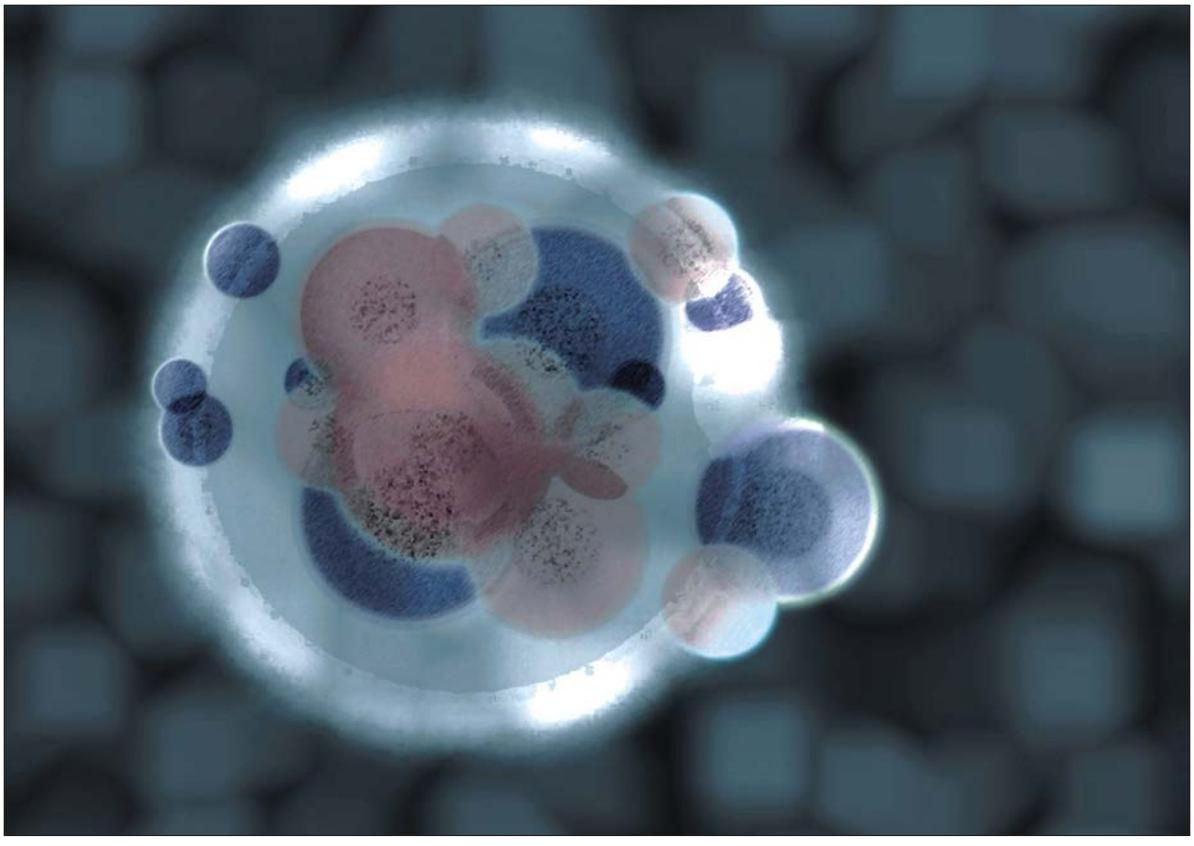

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 11 (8): 475-480, 2011
ISSN 1727-3048 / DOI: 10.3923/jbs.2011.475.480

Flavonoid from *Intsia palembanica* as Skin Whitening Agent

[1,2]I. Batubara, [1,2]L.K. Darusman, [3]T. Mitsunaga, [3]H. Aoki, [1,4]M. Rahminiwati,
[1,5]E Djauhari and [3]K. Yamauchi
[1]Biopharmaca Research Center Bogor Agricultural University,
Jl Taman Kencana No. 3 Bogor, 16151, Indonesia.
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences,
Bogor Agricultural University, IPB Darmaga Campus, Bogor, Indonesia
[3]Faculty of Applied Biological Science, Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan
[4]Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine,
Bogor Agricultural University, Indonesia
[5]Department of Biochemistry, Faculty of Mathematics and Natural Sciences,
Bogor Agricultural University, Indonesia

Abstract: This study aims to obtain active compounds from *Intsia palembanica* with skin whitening activity. *I. palembanica* methanol extracts were separated using chromatography techniques and yielded 3 flavanols. Isolated compounds along with 6 other flavonoid compounds were analyzed for tyrosinase inhibitory activity and inhibition of melanin cell growth in B16 cell. The results showed that (-)-robidanol is the most potent tyrosinase inhibitor (IC_{50} monophenolase 8.7 μM; diphenolase 26.6 μM) and inhibit melanin synthesis 46.2% compared to control (at 100 μM). In conclusion, (-)-robidanol is the best compound as a whitening agent.

Key words: *Instia palembanica*; (-)-robidanol, tyrosinase inhibitor, melanin, whitening agent

INTRODUCTION

Melanin is reported to have a protoprotective function in human skin but humans are still conscious about their skin color because the undesirable skin discoloration or hyperpigmentation can cause aesthetic problems. Because melanin formation is an important factor to human skin color development, the inhibition of melanin formation may result in a reduction of skin darkness. The formation of melanin in the human body is reduced by several mechanisms, including anti-oxidation, direct tyrosinase inhibition, melanin inhibition of migration from cell to cell and hormonal activities, etc (Slominski *et al*., 2004).

Tyrosinase is a rate limiting enzyme associated with melanin synthesis in melanocytes. It is a copper-containing monooxygenase and catalyzes two major reactions: monophenolase, the hydroxylation of L-tyrosine and diphenolase, the oxidation of L-DOPA (3,4-dihydroxyphenylalanine). Inhibition of tyrosinase (monophenolase and diphenolase) activity will decrease the melanin synthesis (Dubey *et al*., 2006).

Present previous research found that out of the 23 plant species screened, *Intsia palembanica* (Local name: Merbau) is a species which has the most potent activity as tyrosinase inhibitor (Batubara *et al*., 2010). *I. palembanica* is extensively used as furniture and building material in place of native wood (Imamura *et al*., 1974). Some ethnic groups in Indonesia use the wood of Merbau as a medicine. For instance, Maluk ethnic group in Sumbawa Island used it to treat impotency (Sangat *et al*., 2000). This plant has only 48.2% survival rate (Hassan *et al*., 2007) and has low growth increments (Affendy *et al*., 2009). It is interesting to know the active compounds in Merbau which has activity as a tyrosinase inhibitor, particularly as skin whitener. Therefore, the aim of this study is to obtain active compounds from Merbau as a skin whitener based on tyrosinase inhibitory activity and melanin cell growth inhibition on B16 cell.

MATERIALS AND METHODS

Plant material: *I. palembanica* was collected from Samarinda, East Kalimantan, Indonesia. Identification and voucher specimen (No. FHT.LA.12.9 p) was deposited at the Wood Anatomy Laboratory, Faculty of Forestry, Mulawarman University, East Kalimantan, Indonesia.

Corresponding Author: L.K. Darusman, Biopharmaca Research Center Bogor Agricultural University,
Jl Taman Kencana No. 3 Bogor, 16151, Indonesia Tel: +62-251-8373561 Fax: +62-251-8347525

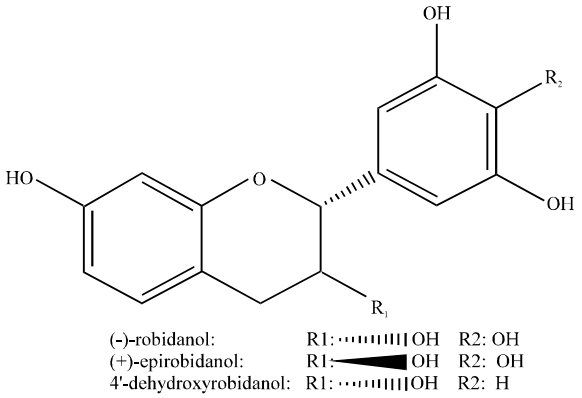

Fig. 1: Structure of Isolated Compounds

Extraction and isolation of (-)-robidanol, (+)-epirobidanol and compound 1: *I. palembanica* was dried and ground before submitting to methanol extraction. Briefly, 100 g *I. palembanica* wood meal was macerated with 5 L methanol for 12 h three times. The extracts were filtered using Whatman filter paper (No. 2) and concentrated *in vacuo* at 30°C using a rotary evaporator to obtain 6.7 g extract (6.7 % yield based on dried sample).

Part of the extract (3 g) was fractionated using n-hexane, EtOAc and water to result to n-hexane soluble part, EtOAc soluble part and aqueous soluble part. EtOAc soluble part (1.4 g) was separated by silica gel column chromatography with hexane, ethyl acetate and methanol as the developing solvents and resulted to 26 fractions. Fraction 4-8 were eluted with ethyl acetate and gave a mixture of (-)-robidanol, (+)-epirobidanol, 4'-dehydroxyrobidanol and some other compounds. Further purification was conducted using preparative HPLC with reversed phase column Inertsil ODS-3 (GL Sciences 10 mm id x 200 mm) monitored at 280 nm. The solvent system used was as follows: a gradient program for 45 minutes from 5 to 100% of methanol in TFA 0.05% (in water) at flow rate of 3 mL min^{-1}. This separation step gave crude (-)-robidanol, (+)-epirobidanol and 4'-dehydroxyrobidanol (Fig. 1). Repeated subjection to preparative HPLC resulted in a (-)-robidanol (15.8 mg), (+)-epirobidanol (47.4 mg) and 4'-dehydroxyrobidanol (10.5 mg). The structures of the compounds were determined by comparison of their spectroscopic data with those reported in the literature. ^{1}H- and ^{13}C- NMR were recorded with a JEOL ECP 600 MHZ spectrometer with TMS as the internal reference and chemical shifts expressed in δ (ppm). Homonuclear ^{1}H-^{1}H COSY and heteronuclear HMBC correlation were analyzed using the same instrument. Mass data was measured by direct injection in Shimadzu GCMS-QP5050A (gas chromatography-mass spectrometer).

Bioactivity tests: Bioactivity tests were performed for 3 isolated compounds and 6 other compounds which were reportedly contained in Merbau such as (+)-catechin, (-)-epicatechin, quercetine, robinetin, ampelopsin and 3,7,3', 5'-tetrahydroxyflavone. Bioactivities were analyzed for tyrosinase activity inhibitor, antioxidants and inhibition of melanin cell growth in B16 cell.

Inhibition of tyrosinase activity (monophenolase) and DOPA auto-oxidation (diphenolase): This assay was performed using methods as described earlier (Curto *et al*., 1999; Nerya *et al*., 2003). Extracts were dissolved in DMSO (dimethyl sulphoxide) to a final concentration of 20 mg mL^{-1}. The extract stock solutions were then diluted to 600 μg mL^{-1} in 50 mM potassium phosphate buffer (pH 6.5).

The extracts were tested at the concentrations ranging from 7.8 to 2000.0 μg mL^{-1}. Kojic acid, which was used as positive control was also tested at concentrations from 7.8 to 500.0 μg mL^{-1}. In 96-well plate, 70 μL of each extract dilution was combined with 30 μL of tyrosinase (333 Units mL^{-1} in phosphate buffer) in triplicate. After incubation at room temperature for 5 min, 110 μL of substrate (2 mM L-tyrosine or 12 mM L-DOPA) was added to each well. Incubation lasted for 30 min at room temperature. Optical densities of the wells were then determined at 510 nm with multi-well plate reader. The concentration of plant extract at which half the original tyrosinase activity is inhibited (IC_{50}), was determined for each plant extract.

Melanin synthesis test on B16 cell

Cell culture: Murine B16 melanoma cells were purchased from Riken Cell Bank (Tsukuba, Japan). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) that was supplemented with 10 % (v/v) fetal bovine serum, 100 Unit mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin, at 37°C in a humidified, CO_2-controlled (5%) incubator.

Melanogenic assays: Cells (4×10^{4} cells) were inoculated on 10 cm dishes and compound treatment began 24 h after seedling. Each compound in DMSO was added to the cell cultures with a final concentration of 0.5% v/v. The cells were harvested 48 h later and the melanin content was determined in triplicate for each treatment (Ohguchi *et al*., 2005). Cells were washed with phosphate-buffered saline (PBS) and dissolved in 1 N NaOH for 1 h at 60°C. The absorbance was measured at 405 nm. The cell proliferation was determined by the trypan blue exclusion test. Cell

proliferation was shown in percentage values. Each percentage value in the treated cells was calculated with respect to that in the control cells.

Statistical analysis: Data of IC_{50} were expressed as Mean±SD. The significant differences between groups were assessed by one-way ANOVA followed by comparisons of the groups with a control using Dunnett's test, $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Identification of compounds: The separation guided by bioassay resulted three isolated compounds from *I. palembanica* namely, (-)-robidanol, (+)-epirobidanol and 4'-dehydroxyrobidanol based on NMR data. The structures of isolated compounds are shown in Fig 1. (-)-robidanol, white powder; ^{1}H-NMR (600MHz, CD_3OD) δ:2.68, 3.06 (each 1H, dd, J = 3.4, 16.5 Hz, H-4), 4.13 (1H, dd, J = 1.9, 6.8 Hz, H-3), 4.79(1H, d, J = 6.8 Hz, H-2), 6.30 (1H, d, J=2.0Hz, H-8), 6.32 (1H, dd, J = 8.2, 2.0Hz, H-6), 6.48 (2H, s, H-2' and H-6'), 6.86 (1H, d, J = 8.2Hz, H-5); ^{13}C-NMR (150MHz, CD_3OD): 32.6 (C-4), 66.5 (C-3), 78.7 (C-2), 102.6 (C-8), 105.6 (C-2' and C-6'),108.2 (C-6), 110.5 (C-4a), 130.1 (C-1'), 130.3 (C-5), 132.3 (C-4'), 145.4 (C-3' and C-5'), 155.2 (C-8a), 156.4 (C-7).; EIMS m/z: 290 [M^+].

(+)-epirobidanol. White powder ^{1}H-NMR (600MHz, CD_3OD): δ:2.64, 2.84 (each 1H, dd, J = 7.6, 15.8Hz, H-4), 3.97 (1H, dd, J = 2.0, 6.9 Hz, H-3), 4.60 (1H, d, J = 6.9Hz, H-2), 6.26 (1H, d, J = 2.1Hz, H-8), 6.30 (1H, dd, J = 8.2, 2.1Hz, H-6), 6.36 (2H, s, H-2' and H-6'), 6.83 (1H, d, J = 8.2Hz, H-5); ^{13}C-NMR (150 MHZ, CD_3OD): 31.3 (C-4), 67.5 (C-3), 81.6 (C-2), 102.3 (C-8), 105.7 (C-2' and C-6'), 108.1 (C-6), 111.2 (C-4a), 129.9 (C-5), 130.3 (C-1'), 130.0 (C-5), 132.7 (C-4'), 145.6 (C-3' and C-5'), 154.8 (C-8a), 156.6 (C-7). EIMS m/z: 290 [M^+].

4'-dehydroxyrobidanol. White powder, ^{1}H-NMR (600 MHZ, CD_3OD): δ: 2.69, 3.08 (each 1H, dd, J = 2.8, 13.7Hz, H-4), 4.20 (1H, dd, J = 1.9, 6.8 Hz, H-3), 4.56 (1H, d, J = 1.9 Hz, H-2), 6.18(1H, t, J = 2.0, H-4'), 6.32 (1H, dd, J = 2.0, 8.2Hz, H-6), 6.33 (1H, d, J = 2.0Hz, H-8), 6.43 (2H, d, J = 2.0Hz, H-2'& 6'), 6.80 (1H, d, J = 8.2Hz, H-5); ^{13}C-NMR (150 MHZ, CD_3OD): 32.6 (C-4), 66.4 (C-3), 78.0 (C-2), 101.4 (C-4'), 102.7 (C-8), 108.3 (C-6), 104.9 (2C, C-2' and 6'), 109.0 (C-4a), 130.3 (C-5), 141.0 (C-1'), 155.0 (C-8a), 156.0 (C-7), 157.0 (2C, C-3' and 5'); EIMS m/z: 274[M^+]

Structure of three compounds were proved by homonuclear corellation data (COSY) and heteronuclear corellation data (HMBC). The importance data was found in HMBC NMR data. The HMBC data was especially usefull in determining the placement of B-ring. Proton from B-ring (H-2' and 6') were correlated to carbon no 2 in C-ring and also correlated to carbon No. 1', 2', 3', 4', 5' and 6' in B-ring.

Table 1: Tyrosinase IC_{50} values of pure compounds from *I. palembanica*

Compound name	IC_{50} (μM) monophenolase*	IC_{50} (μM) diphenolase*
(-)-robidanol	8.7±1.9^a	26.6±2.4^a
(+)-epirobidanol	20.2±1.9^c	178.5±19.1^d
4'-dehydroxyrobidanol	15.2±1.6^b	50.0±8.4^b
robinetin	1046.9±112.4^h	-
3,7,3',5'-tetrahydroxyflavone	-	-
Ampelopsin	59.4±9.9^d	5482.2±234.5^e
Quercetin	81.3±4.9^f	-
(+)-catechin	76.6±2.7^e	-
(-)-epicatechin	92.5±3.1^g	-
Kojic acid	55.6±2.2^d	126.1±11.2^c

*Data given as mean±standard deviation of triplicate tests. IC_{50} data followed by the same letter are not significantly different according to Dunnett's test, $p<0.05$. Filed to achieve 50% inhibition at maximum concentration of 2500 μg mL^{-1}

Bioactivity tests: The tyrosinase inhibitory activity of pure compounds, 6 pure compounds reported in *I. palembanica* and positive control (kojic acid) are shown Table 1. The results showed that (-)-robidanol was the most active compounds with IC_{50} (monophenolase: 8.7 μM and diphenolase: 26.6 μM). 4'-dehydroxyrobidanol was the next prospective compounds with IC_{50} (monophenolase: 15.2 μM and diphenolase: 50.0 μM). These two isolated compounds had activity better than kojic acid as positive control.

Almost all of the compounds reached IC_{50} before the maximum concentration 2500 μg mL^{-1}. Only 3,7,3',5'-tetrahydroxyflavone failed to achieve 50% inhibition at maximum concentration. For diphenolase activity, only 4 compounds achieved 50% inhibition ((-)-robidanol, (+)-epirobidanol, 4'-dehydroxyrobidanol and ampelopsin).

The result of melanin synthesis inhibitor is showed Fig. 3. The three isolated compounds had cell viability between 95-140% which not significantly different compared to cell in 0.5% DMSO. Melanin synthesis activities of the three isolated compounds were depended on their concentration. (+)-epirobidanol had inhibition activity at high concentration (100 μM), while at lower concentration had acceleration activity (1.6 μM). (-)-robidanol gave melanin synthesis inhibition activity at all concentration applied. Different with the two isolated compounds, 4'-dehydroxyrobidanol gave melanin acceleration activity at all concentration applied.

Isolated compounds from *I. palembanica*: Bioassay-guided separation of Merbau extracts led to the purification of 3 compounds. Three flavanol compounds were isolated: (-)-robidanol, (+)-epirobidanol and 4'-dehydroxyrobidanol. (-)-robidanol and (+)-epirobidanol

are flavanol compounds which had been found in some fruits, like *Gleditschia triacanthos* (Weinges, 1964), strawberry (Lopes-da-Silva *et al*., 2007), red grape (Makris *et al*., 2008) and *Annona senegalensis* (Potchoo *et al*., 2008).

Among all the flavanol which isolated, 4'-dehydroxyrobidanol was found to be a novel compound and its structure was elucidated by NMR data. 4'-dehydroxyrobidanol gave molecular ion peak at m/z 274 [M^+], consistent with molecular formula of $C_{15}H_{14}O_5$. In its proton NMR spectrum, the signal for ring A and the aliphatic signal were the same with (-)-robidanol. The differences were found only in signal of ring B. It has signal δ at 6.18 ppm with *J* about 2 Hz and coupling with 2 proton signals δ at 6.43 ppm with the same coupling constant. The data also proved by proton-proton correlation (COSY) data. This data mean that B rings had no hydroxyl group at C-4'. It is an unusual flavonoids pattern especially in ring B but the same pattern had been reported to exist in the same species of *I. palembanica* (Imamura *et al*., 1974). They reported a new type of flavonol in the shake of Merbau wood. The new type flavonol was 3,7,3',5'-tetrahydroxyflavone, a pale yellow microcrystals.

Other flavonoids isolated from Merbau belong to 2 groups, flavanols and flavonols. The flavonoid belonging to flavanol group were (+)-catechin and (-)-epicatechin while those belonging to the flavonol group were robinetin, myricetin, fisetin, quercetin, naringenin, ampelopsin, leucocyanidin and piceatannol. On this paper, we used robinetin, ampelopsin, 3,7,3',5'-tetrahydroxyflavone which were isolated previously together with quercetin, (+)-catechin and (-)-epicatechin from Merck for the activity tests (Fig. 2c, b).

Tyrosinase inhibitory activity: The tyrosinase inhibitory activity of pure compounds is shown in Table 1 with kojic acid as positive control. The data reported in IC_{50} values are the concentrations which can inhibit 50% activity compared to the control. The results showed that isolated flavanol compounds were more active to inhibit tyrosinase activity compared to flavonol compounds either in monophenolase or diphenolase.

Many reported flavonoid compounds had the capability to inhibit tyrosinase activity but those of compounds from the flavanol group were still limited. (-)-epigallocatechin is a compound from the flavanol group which has been reported to have a monophenolase inhibitory activity (IC_{50} 0.035 mM) (No *et al*., 1999). A study about the flavanol group reported (+)-catechin as a cofactor or substrate for tyrosinase (Kubo *et al*., 2000). Present results showed that the IC_{50} of (-)-epicatechin was

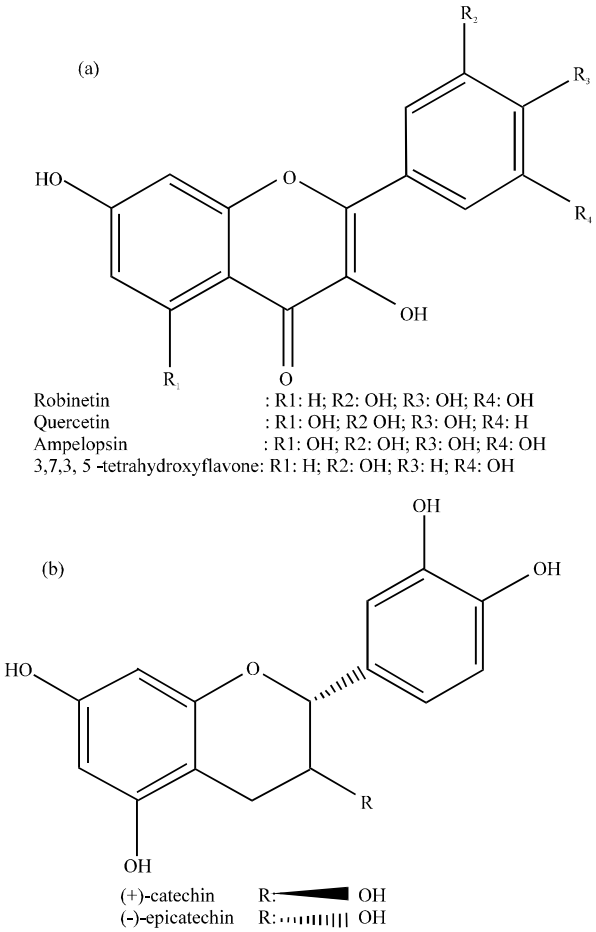

Fig. 2(a-b): Flavonol and catechin compounds reportedly isolated from *I. palembanica*

about 0.092 mM for monophenolase and (+)-catechin inhibited the tyrosinase activity for monophenolase with IC_{50} of about 0.077 mM.

Compared to (+)-catechin and (-)-epicatechin which only had monophenolase inhibition activity, (-)-robidanol, (+)-epirobidanol and 4'-dehydroxyrobidanol had inhibition activities against tyrosinase either for monophenolase or diphenolase. The most active tyrosinase inhibitor found on (-)-robidanol with IC_{50} for monophenolase of about 8.7 μM and diphenolase about 26.6 μM. Its activity is about 4 times compared to kojic acid as positive control. When we compared the chemical structure of (-)-robidanol and (-)-epigallocatechin, (-)-robidanol has no hydroxyl group on C-5. This data indicated the absence of hydroxyl group on C-5, probably can increase the tyrosinase inhibitory activity. The other interesting data was the absence of hydroxyl groups on C-4' of 4'-dehydroxyrobidanol which can decrease the tyrosinase inhibitory activity about two times compared to (-)-robidanol.

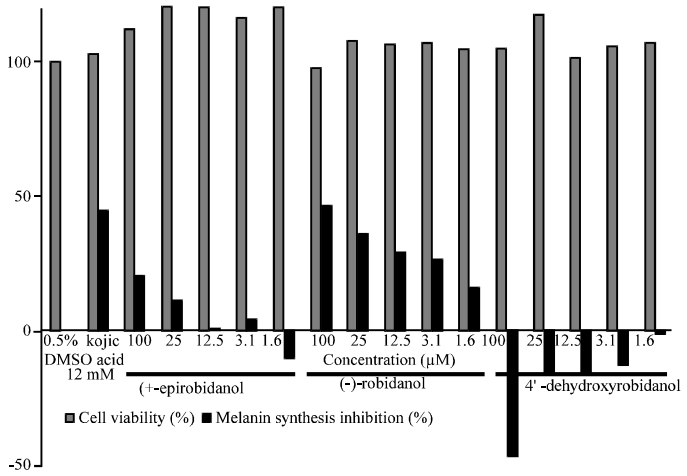

Fig. 3: Cell viability and % melanin synthesis of isolated compounds

For flavonol groups found in *I. palembanica*, the most active flavonol compound was ampelopsin which had IC_{50} value not significantly different with kojic acid as positive control on monophenolase activity. The other flavonol compounds did not show IC_{50} data on diphenolase. Interestingly, 3,7,3‘,5‘-tetrahydroxyflavone did not show inhibition activity for tyrosinase for both monophenolase and diphenolase, while 4‘-dehydroxyrobidanol which has the same B-ring pattern showed some inhibition activity.

Melanin synthesis on B16 cell and cell viability: Another approach for a whitening agent is to inhibit melanin synthesis and it called as melanogenesis. Kim *et al.* (2010) stated that melanogenesis is a well known physiological response of human skin which mainly caused by ultraviolet irradiation and some genetic factors. Many natural products searched for anti-melanogenesis such as from *Neolitsea aciculate* (Kim *et al.*, 2010) and sea cucumber (*Stichopus japonipus*) (Yoon *et al.*, 2010).

Our research focus on the compounds isolated from *I. palembanica*. Based on the color of cell which subjected with (-)-robidanol was lighter compared to blank cell. Compared with (-)-robidanol, 4’-dehydroxyrobidanol cannot change the color of cell. It can be concluded that 4’-dehydroxyrobidanol cannot inhibit the synthesis of melanin.

The melanin synthesis inhibition and cell viability of B16 cell in the presence of isolated compounds are shown in Fig. 3. All the isolated compounds were not toxic to the cell because cell viability is almost the same with the blank and a little bit increasing compared to blank cell (Fig. 3). This result showed that the compounds were safe to use for cell assay

The melanin synthesis inhibition of each compound was different from each other. (-)-robidanol still showed the most active compound as a whitening agent with the melanin synthesis inhibition approach. (-)-robidanol inhibit melanin synthesis of about 46.2% at 100 μM concentration. This activity was higher compared to kojic acid as positive control which only inhibited about 44.0 % at 12 mM concentration.

(+)-epirobidanol at 100 μM concentration had the activity to inhibit the melanin synthesis of about 21.0% or about 45.0% activity of (-)-robidanol. The difference in the activity may due to the differential activity of each compound to inhibit tyrosinase. Compared to (+)-epirobidanol and (-)-robidanol, 4‘-dehydroxyrobidanol did not inhibit melanin synthesis. 4‘-dehydroxyrobidanol increased the melanin synthesis on B16 cell of about 47.0%.

CONCLUSION

Three flavanol compounds were isolated from *Intsia palembanica*, namely (-)-robidanol, (+)-epirobidanol and 4’-dehydroxyrobidanol. Isolated compounds along with 6 other flavonoid compounds were analyzed for tyrosinase inhibition and inhibition of melanin cell growth in B16 cell. (-)-Robidanol was the best compound as a whitening agent based on tyrosinase and melanin synthesis inhibitory activities.

ACKNOWLEDGMENT

We express our gratitude to the Faculty of Forestry Mulawarman University, East Kalimantan, Indonesia for preparation of the samples. We also thank Ms. Mary

Grace Saldajeno for grammatical correction of the manuscript. This work was supported by Higher Education Directorate of the National Education Department of Republic of Indonesia (Hibah kompetitif penelitian untuk publikasi internasional No: 4472H/PP/DP2M/VI/2010) and the Chemistry of Natural Products Laboratory, Gifu University, Japan.

REFERENCES

Affendy, H., M. Aminuddin. W. Razak, A. Arifin and A.R. Mojiol, 2009. Growth increments of indigenous species planted in secondary forest area. Res. J. For., 3: 23-28.

Batubara, I., L.K. Darusman, T. Mitsunaga, M. Rahminiwati and E. Djauhari, 2010. Potency of Indonesian medicinal plants as tyrosinase inhibitor and antioxidant agent. J. Biol. Sci., 10: 138-144.

Curto, E.V., C. Kwong, H. Hermersdorfer, H. Glatt and C. Santis *et al*., 1999. Inhibitors of mammalian *Melanocyte tyrosinase*: *In vitro* comparisons of alkyl esters of gentisic acid with other putative inhibitors. Biochem. Pharmacol., 57: 663-672.

Dubey, S.K., K. Misra, A. Tiwari and A.K. Bajaj, 2006. Chemically induced pigmentary changes of human skin, interaction of some azo dyes with human DNA. J. Pharmacol. Toxicol., 1: 234-247.

Hassan, A., R. Wahab, M.A. Alias and RM. Salim, 2007. Growth performance of 9-years-old selected 5 indigenous wood species planted on degraded forest land. Int. J. Agric. Res., 2: 302-306.

Imamura, H., K. Nomura, Y. Hibino and H. Ohashi, 1974. A new flavonol in the shake of Merbau wood. Mokuzai Gakkaishi, 20: 143-144.

Kim, S.S., W.J. Yoon, C.G. Hyun and N.H. Lee, 2010. Down-regulation of tyrosinase, TRP-2 and MITF expressions by *Neolitsea aciculata* extract in murine B16 F10 melanoma. Int. J. Pharmacol., 6: 290-295.

Kubo, I., I. Kinsy-Hori, J.K. Chaudari, Y. Kubo, Y. Sandoz and T. Ogura, 2000. Flavonols from *Heterotheca inuloides*: Tyrosinase inhibitory activity and structural critera. Bioorg. Med. Chem., 8: 1749-1755.

Lopes-da-Silva, F., M.T. Escribano-Baion and C. Santos-Buelga, 2007. Stability of pelargonidin 3-glucoside in model solutions in the presence and absence of flavanols. Am. J. Food Technol., 2: 602-617.

Makris, D.P., G. Boskou, A. Chiou and N.K. Andrikopoulos, 2008. An investigation on factors affecting recovery of antioxidant phenolics and anthocyanins from red grape (*Vitis vinifera* L.) pomace employing water/ethanol-based solutions. Am. J. Food Technol., 3: 164-173.

Nerya, O., J. Vaya, R. Musa, S. Izrael, R. Ben-Arie and S. Tamir, 2003. Glabrene and isoliquiritigenin as tyrosinase inhibitors from Liquorice roots. J. Agric. Food. Chem, 51: 1201-1207.

No, J.K., D.Y. Soung, Y.J. Kim, K.H. Shim and Y.S. Jun *et al*., 1999. Inhibition of tyrosinase by green tree components. Life Sci., 65: 241-246.

Ohguchi, K., Y. Akao and Y. Nozawa, 2005. Involvement of calpain in melanogenesis of mouse B16 melanoma cells. Mol. Cell. Biochem., 275: 103-107.

Potchoo, Y., I.P. Guissou, M. Lompo, E. Sakie and B. Yaro, 2008. Antioxidant activity of aqueous methanol and ethyl acetate extract of leaves of *Annona senegalensis* Pers from togo versus the one originates from Burkina Faso. Int. J. Pharmacol., 4: 120-124.

Sangat, H.M., E.A.M. Zuhud and E.K. Damayanti, 2000. Kamus Penyakit dan Tumbuhan Obat Indonesia: Etnofitomedika. Volume 1, Yayasan Obor Indonesia, Jakarta, ISBN: 979-461-364-9, Pages: 209.

Slominski, A., J.T. Desmond, S. Shibahara and J. Wortsman, 2004. Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol. Rev., 84: 1155-1228.

Weinges, K., 1964. The occurrence of catechins in fruits. Phytochemistry, 31: 263-266.

Yoon, W.J., M.J. Kim, H.B. Koh, W.J. Lee, N.H. Lee and C.G. Hyun, 2010. Effect of korean red sea cucumber (*Stichopus japonicus*) on melanogenic protein expression in murine B16 melanoma. Int. J. Pharmacol., 6: 37-42.