Antioxidant and Antimelanogenesis Activities of Glyasperin A From Macaranga pruinosa Leaves

by Enih Rosamah

Submission date: 27-Aug-2021 11:22AM (UTC+0700)
Submission ID: 1636637810
File name: 13._Antioxidant_and_Antimelanogenesis_Activities.pdf (309.16K)
Word count: 2833
Character count: 15082

Check for updates

Short Communication

Natural Product Communications
July 2019: 1–5
© The Author(s) 2019
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1934578X19867192
journals.sagepub.com/home/npx

SAGE

Antioxidant and Antimelanogenesis Activities of Glyasperin A From *Macaranga pruinosa* Leaves

Enos T. Arung[1,2], Jhonner R. Sinamabela[1], Enih Rosamah[1], Irawan W. Kusuma[1,2], Harlinda Kuspradini[1,2], Ahmed E. Alam[3,4], Yhiya Amen[3,5], Hiroyuki Tanaka[6], Dedi Satria[3,7], Kuniyoshi Shimizu[3], and Hiroya Ishikawa[8]

Abstract

In our effort to find materials for drugs and cosmetics from tropical natural resources, we screened 21 methanol extracts from 7 *Macaranga* trees species (*Macaranga bancana, Macaranga gigantea, Macaranga hullettii, Macaranga pruinosa, Macaranga tanarius, Macaranga trichocarpa, and Macaranga triloba*) for antioxidant and antimelanogenesis. The antioxidant and melanogenesis (tyrosinase enzyme assay and melanin inhibitor in B16 melanoma) assays were used to determine the activities. The fractionation and the isolation of active compound were done by various chomatographic methods and the structure was determined by spectroscopic analysis data. We isolated a prenylated flavonoid, named Glyasperin A, from *M. pruinosa* leaf. This compound showed a potency as antioxidant and inhibited melanin in B16 melanoma but not tyrosinase activity. These results showed that the methanol leaf extracts of *M. pruinosa* could be developed for cosmetic applications especially as a skin whitening agent.

Keywords

Macaranga trees, *Macaranga pruinosa*, flavonoid, antioxidant, antimelanogenesis

Received: April 24th, 2019; Accepted: July 5th, 2019.

In search of material for drugs and cosmetics, we have screened 7 species of *Macaranga* trees (*Macaranga bancana, Macaranga gigantea, Macaranga hullettii, Macaranga pruinosa, Macaranga tanarius, Macaranga trichocarpa, and Macaranga triloba*) in cancer (MCF-7, B16 melanoma, HCT116, and Hela) and normal (TIG-1 and NHDF) cells, previously.[1] *Macaranga pruinosa* is a tree with up to 15 m of height, diameter of breast height up to 20 cm. The bark is smooth, hooped, whitish-gray, and with red sap. The inner bark is reddish-brown and granular, the male flowers are pale green, and the fruits are green.[2] The previous biological or chemical studies on *M. pruinosa* have shown antioxidant, antityrosinase, anticholinesterase, nitric oxide inhibition, and antibacterial activities of leaf extracts[3,4] and chemical compounds such as flavonoids (macapruinosin B, C, D, E, F and Nymphaeol C)[5,6] and Stilbene (Macapruinosin A) were produced.[5] The young shoots' extracts of *M. gigantea, M. pruinosa, and M. triloba* are used for treating fungal infections and the leaves for treating stomach aches.[7] In this study, we reported the antioxidant and antimelanogenesis (antityrosinase and antimelanin) of the extract and the isolated compound.

Results and Discussion

The reactive oxidants such as reactive oxygen species (ROS) and H_2O_2 are produced in melanogenesis, which create oxidative

[1]Laboratory of Forest Product Chemistry, Faculty of Forestry, Mulawarman University, Samarinda, Indonesia
[2]Research Center for Medicine and Cosmetic from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia
[3]Department of Agro-Environmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka, Japan
[4]Department of Pharmacognosy and Medicinal Plant, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt
[5]Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Egypt
[6]Department of Medicinal Resources Regulation, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan
[7]Faculty of Health and Sciences, Muhammadiyah University of Sumatera Barat, Bukittinggi, Indonesia
[8]Department of Food and Health Sciences, International College of Arts and Science, Fukuoka Women's University, Japan

Corresponding Authors:
Enos T. Arung, Laboratory of Forest Product Chemistry, Faculty of Forestry, Mulawarman University, Jl. KH Dewantara, Kampus Gn. Kelua, Samarinda, East Kalimantan 75123, Indonesia.
Email: tangkearung@yahoo.com
Hiroya Ishikawa, Department of Food and Health Sciences, International College of Arts and Science, Fukuoka Women's University, Fukuoka, Japan.
Email: ishikawa@fwu.ac.jp

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Table 1. Effect of *Macaranga* Trees Extracts on Antioxidant, Tyrosinase Enzyme Activity, and Melanin Inhibition in B16 Melanoma Cells at 100 mg/mL.

		DPPH[a] activity	Tyrosinase activity[a]	B16 melanoma[a]	
Samples	Parts	(%)	(%)	Melanin (%)	Cell viability (%)
Macaranga bancana	Bark	25.54 ± 0.004	109.80 ± 0.013	117.9 ± 0.010	105.30 ± 0.090
	Wood	22.71 ± 0.007	119.10 ± 0.006	110.10 ± 0.030	110.20 ± 0.140
	Leaf	36.05 ± 0.003	98.30 ± 0.005	78.80 ± 0.020	53.00 ± 0.140
Macaranga gigantea	Bark	27.80 ± 0.004	116.40 ± 0.010	77.50 ± 0.020	121.10 ± 0.030
	Wood	27.80 ± 0.004	114.50 ± 0.004	136.90 ± 0.020	94.60 ± 0.040
	Leaf	52.32 ± 0.002	97.80 ± 0.040	77.90 ± 0.020	50.40 ± 0.040
Macaranga hullettii	Bark	40.45 ± 0.010	93.20 ± 0.007	130.90 ± 0.020	121.40 ± 0.080
	Wood	22.37 ± 0.002	111.10 ± 0.006	143.10 ± 0.020	90.40 ± 0.010
	Leaf	28.59 ± 0.003	99.20 ± 0.010	109.70 ± 0.008	121.90 ± 0.180
Macaranga pruinosa	Bark	9.72 ± 0.006	119.80 ± 0.030	150.10 ± 0.010	148.60 ± 0.030
	Wood	2.06 ± 0.004	94.90 ± 0.020	109.20 ± 0.020	131.90 ± 0.010
	Leaf	37.18 ± 0.005	73.50 ± 0.070	24.10 ± 0.005	17.80 ± 0.010
Macaranga tanarius	Bark	28.93 ± 0.001	89.10 ± 0.010	121.90 ± 0.010	109.00 ± 0.240
	Wood	44.29 ± 0.01	94.30 ± 0.009	172.30 ± 0.020	106.00 ± 0.030
	Leaf	26.89 ± 0.002	113.30 ± 0.010	30.70 ± 0.005	11.10 ± 0.020
Macaranga trichocarpa	Bark	36.16 ± 0.003	107.80 ± 0.006	143.60 ± 0.020	141.20 ± 0.110
	Wood	23.50 ± 0.007	91.90 ± 0.010	139.20 ± 0.010	116.60 ± 0.120
	Leaf	36.84 ± 0.006	105.80 ± 0.003	38.40 ± 0.004	34.60 ± 0.230
Macaranga triloba	Bark	24.41 ± 0.001	115.30 ± 0.010	111.60 ± 0.050	100.40 ± 0.060
	Wood	40.11 ± 0.005	119.80 ± 0.010	163.70 ± 0.040	105.50 ± 0.070
	Leaf	60.57 ± 0.01	93.80 ± 0.020	186.20 ± 0.020	127.90 ± 0.080
Ascorbic acid		3.96 ± 0.001	-	-	-
Kojic acid		-	5.40 ± 0.001	-	-
Arbutin		-	-	58.70 ± 0.001	96.40 ± 0.060

DPPH, diphenyl-2-picrylhydrazyl.
Data are represented as the mean ± SD ($n = 3$)
[a]Percentage vs control.

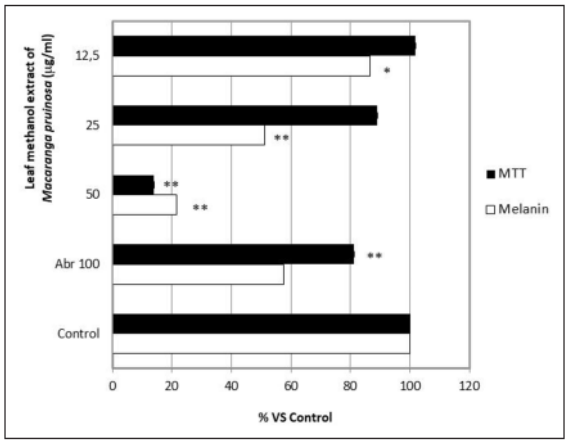

Figure 1. Antimelanogenesis in B16 melanoma cells of *Macaranga pruinosa* leaf extracts (all values: mean ± SD; Arb 100, Arbutin 100 µg/mL). Significantly different from the control value (Student's *t* test) : $P < 0.05$ (*) and $P < 0.01$ (**).

stress in melanocytes. Some ROS scavengers and inhibitors of ROS generation inhibit UV-induced melanogenesis, and antioxidants like reduced glutathione and ascorbic derivatives are applied to treat various skin problems such as hyperpigmentation. Hence, antioxidants regulate the important role in hyperpigmentation or melanogenesis.[8] In this study, we have screened those extracts for antioxidants and antimelanogenesis as seen in Table 1 in order to evaluate its potency as cosmetic ingredient. Based on Table 1 , Figure 1, and thin layer chromatography (TLC), the leaf extract of *M. pruinosa* was chosen for further fractionation to isolate the active compound. A total of 27 fractions were collected and their antioxidant activities were determined. Fraction 11 was the potent fraction for antioxidant and focused on the isolation of the active compound. By using some chromatographic methods and nuclear magnetic resonance (NMR) assigments (^{1}H, ^{13}C, distortionless enhancement by polarization transfer [DEPT], heteronuclear single quantum coherence [HSQC], and heteronuclear multiple bond correlation [HMBC]), Glyasperin A[9,10] was obtained.

Next, we determined antioxidant and antimelanogenesis activities of Glyasperin A. Table 2 depicts the antioxidant

Table 2. Antioxidant Activities of Isolated Compound (Glyasperin A) From *Macaranga pruinosa* Leaf Extracts (All Values: Mean ± SD).

Compound	DPPH IC_{50}(μM/mL)	ABTS IC_{50}(μM/mL)	SOSA IC_{50}(μM/mL)	ORAC (μmol TE/mg)
Glyasperin A	443.0 ± 8.0	210.0 ± 2.7	308.0 ± 23.3	2.5 ± 0.6
Kaempferol	238.0 ± 3.3	111.0 ± 1.6	47.0 ± 10.0	34.9 ± 1.9

DPPH, diphenyl-2-picrylhydrazyl; ORAC, oxygen radical absorbance capacity; SOSA, superoxide dismutase-like activity.

activities such as diphenyl-2-picrylhydrazyl (DPPH), (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) [ABTS], superoxide dismutase-like activity (SOSA), and oxygen radical absorbance capacity (ORAC). All the IC_{50} of Glyasperin A showed almost a half lower than the positive control (Kaempferol), excepted ORAC activity which was stronger. In Figure 2, Glyasperin A showed a potency for antimelanogenesis, especially inhibited melanin in B16 melanoma cells but less cytotoxicity to the cells. Glyasperin A inhibited melanin in B16 melanoma cells at concentration 1 to 2 μg/mL up to 40% but less cytotoxicity (<7%) which is better than arbutin as positive control. Unfortunately, Glyasperin A did not inhibit tyrosinase enzyme activity (data not shown). Based on the above results, Glyasperin A could be a candidate for cosmetic applications especially as a skin whitening material with antioxidant capacity. Further experiments need to address the mechanism of melanogenesis and its safety for human use.

Experimental

General

The ^{1}H, ^{13}C, DEPT, HSQC, and HMBC NMR were recorded on a Bruker DRX 600 NMR (Bruker Daltonics, Billerica, MA, United States). High resolution electrospray ionisation mass spectrometry (HR-ESI-MS) was determined with liquid chromatograph mass spectrometer ion trap time of flight (LC-MS-IT-TOF) (Shimadzu, Tokyo, Japan). The silica gel column chromatography was conducted using Wakogel C-200. The preparative high-performance liquid chromatography (HPLC) column, Inertsil Prep-octadecyl silica (ODS) (20 mm i.d. × 250 mm, GL-Science) was used. UV spectra were recorded on JASCO-V-530 spectrophotometer. All chemicals were commercially available, such as methanol (MeOH) (Wako, Japan), DPPH and dimethylsulfoxide (DMSO) (Wako, Japan), ethylenediaminetetraacetic acid (Dojindo, Japan), fetal bovine serum (FBS), and Eagle's minimum essential medium (EMEM, Gibco, United States). The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide was from Sigma (United States).

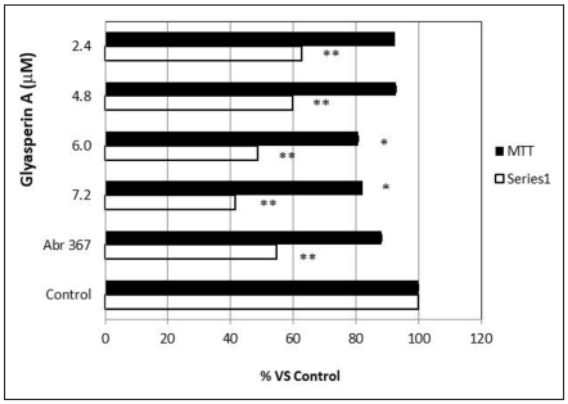

Figure 2. Antimelanogenesis in B16 melanoma cells of Glyasperin A (all values: mean ± SD; Arb 367, Arbutin 367 μM). Significantly different from the control value (Student's *t* test) : $P < 0.05$ (*) and $P < 0.01$ (**).

Plant Materials

All *Macaranga* trees were collected in November 2015 from the Forest Education of Mulawarman University, Samarinda, East Kalimantan, Indonesia. The specimen were identified by Raharjo S.Hut and deposited in the Forest Products Chemistry Laboratory, Department of Forest Product Technology, Faculty of Forestry, Mulawarman University. The plant parts (wood, leaf, and bark) were dried, powdered, and extracted (each 50 g) with 360 mL of MeOH at room temperature for 48 hours. The extract solutions were filtered and concentrated to gain the methanol extracts. The leaves of *M. pruinosa* were recollected in May 2016 in Samarinda, Indonesia.

Extraction, Fractionation, and Isolation of Active Compound

The leaf *M. pruinosa* (1 kg) was re-extracted into MeOH at 72 hours in room temperature and yielded 128.02 g. About 10 g of extracts were fractionated with silica gel column chromatography (130 g of Wakogel C-200, 4.14 × 50 cm) and eluted with *n*-hexane/EtOAc in the ratio of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10 and EtOAc/MeOH in the ratio of 8:2, 6:4, 4:6, 2:8, and 0:10 to give 27 fractions (F1-F27). Based on antioxidant (DPPH), tyrosinase activity, TLC, and HPLC results, F11 (204 mg) was subjected to isolate the active compound by preparative HPLC (Inertsil Prep-ODS:20 mm i.d. × 250 mm). Isolation process was eluted with MeOH/H_2O (0.1% trifluoroacetic acid, TFA), 95:5, 5

mL/min, yielded 3 fractions and fraction F11-3 (57.5) was focused for purification. The fraction F11-3 was repreparative HPLC with MeOH/H_2O (0.1% TFA), 75:25, 5 mL/min to obtain Glyasperin A (8.1 mg).

Antioxidant Assays

Radical Scavenging (Diphenyl-2-Picrylhydrazyl)

The sample was dissolved in DMSO and used for the actual experiment at 30 times dilution. The assay was performed as previously described.[11] Ascorbic acid/Vitamin C and Kaempferol were used as a positive control.

Oxygen Radical Absorbance Capacity

Samples were directly dissolved in acetone/water/acetic acid (70:29.5:0.5, v/v/v) and diluted with 75 mM potassium phosphate buffer (pH 7.4) for analysis. Trolox, fluorescein sodium (FL), and (2,2'-Azobis(2-amidinopropane) dihydrochloride) [AAPH] solutions were prepared with 75 mM phosphate buffer (pH 7.4). The ORAC assay was performed as described by Ou et al[12] and Kaempferol was used as a positive control.

ABTS Radical Cation Decolorization

The working solution of ABTS was prepared with 5 mL of 7 mM ABTS solution and 88 µL of 140 mM potassium persulfate. The ABTS assay was performed as described previously.[13] The results were calculated in the same way as for the DPPH and expressed in terms of Trolox equivalent antioxidant capacity (TEAC µg/mg). Kaempferol was used as a positive control.

Superoxide Dismutase-Like Activity

Samples were added to the (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) [WST] working solutions (200 µL) containing 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium in 50 mM carbonate buffer (pH 10.2). This assay was performed as described previously[13] and Kaempferol was used as a positive control.

Tyrosinase Enzyme Assay

Although mushroom tyrosinase differs somewhat from the other sources, this fungal source was used for the present experiment due to its ready availability. The tyrosinase activity was determined with the method as previously described.[11] Kojic acid was used as a positive control.

Determination of Melanin Content and Cell Viability in B16 Melanoma Cells

A mouse melanoma cell line, B16, was obtained from RIKEN Cell Bank. The cells were maintained in EMEM supplemented with 10% (v/v) FBS and 0.09 mg/mL theophylline. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2. These assays for determining melanin content and cell viability were determined as described by Arung et al.[11] Arbutin was used as a positive control.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was financially supported by Islamic Development Bank Grant for Mulawarman University (No. 137/UN17.11/PL/2019).

ORCID ID

Yhiya Amen https://orcid.org/0000-0003-2552-4567

References

1. Arung ET, Amirta R, Zhu Q, Amen Y, Shimizu K. Effect of wood, bark and leaf extracts of Macaranga trees on cytotoxic activity in some cancer and normal cell lines. *J Indian Acad Wood Sci.* 2018;15(2):115-119.
2. Slick JWF. Keys to the taxa of Macaranga and Mallotus (Euphorbiaceae) of East Kalimantan (Indonesia). *Flora Malesiana Bull.* 1998;12:157-178.
3. Mazlan NA, Mediani A, Abas F, et al. Antioxidant, Antityrosinase, Anticholinesterase, and Nitric Oxide inhibition activities of three Malaysian *Macaranga* species. *Scientific World J.* 2013;2013(1):1-8.
4. Lim TY, Lim YY, Yule CM. Bioactivity of leaves of *Macaranga* species intropical peat swamp and non-peat swamp environments. *J Trop For Sci.* 2014;26(1):134-141.
5. Syah YM, Ghisalberti EL. Phenolic derivatives with an irregular sesquiterpenyl side chain from *Macaranga pruinosa. Nat Prod Commun.* 2010;5(2):219-222.
6. Syah YM, Ghisalberti EL. More phenolic derivatives with an irregular sesquiterpenyl side chain from *Macaranga pruinosa. Nat Prod J.* 2012;2(1):45-49.
7. Grosvenor PW, Supriono A, Gray DO. Medicinal plants from Riau province, Sumatra, Indonesia. Part 2: antibacterial and antifungal activity. *J Ethnopharmacol.* 1995;45(2):97-111.
8. Kim M, Shin S, Lee JA, Park D, Lee J, Jung E. Inhibition of melanogenesis by *Gaillardia aristata* flower extract. *BMC Complement Altern Med.* 2015;15(1):449-460.
9. Zenq L, Fukai T, Nomura T, Zhang RY, Lou ZC. Four new prenylated flavonoids, glyasperins A, B, C, and D from the roots of *Glycyrrhiza aspera. Heterocycles.* 1992;34:575-587.
10. Aminah NS, Kristanti AN, Tanjung M. Antioxidant activity of flavonoid compounds from the leaves of *Macaranga gigantea. J Chem Pharmaceu Res.* 2014;6(6):688-692.
11. Arung ET, Suwinarti W, Hendra M, et al. Determination of antioxidant and anti-melanogenesis activities of durio kutejensis

[Bombacaceae (Hassk.) Becc] (Indonesian Lai) fruit extract. *Trop J Pharm Res*. 2015;14:41-46.

12. Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J Agric Food Chem*. 2001;49(10):4619-4626.
13. Tanaka A, Zhu Q, Tan H, et al. Biological activities and phytochemical profiles of extracts from different parts of bamboo (*Phyllostachys pubescens*). *Molecules*. 2014;19(6):8238-8260.

Antioxidant and Antimelanogenesis Activities of Glyasperin A From Macaranga pruinosa Leaves

ORIGINALITY REPORT

12%	12%	1%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	hyoka.ofc.kyushu-u.ac.jp Internet Source	2%
2	1library.net Internet Source	2%
3	coek.info Internet Source	1%
4	docplayer.net Internet Source	1%
5	repository.naturalis.nl Internet Source	1%
6	www.jocpr.com Internet Source	1%
7	www.scielo.br Internet Source	1%
8	kyushu-u.pure.elsevier.com Internet Source	1%
9	www.alliedacademies.org Internet Source	1%

10	link.springer.com Internet Source	1%
11	www.nature.com Internet Source	1%
12	innovareacademics.in Internet Source	<1%

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	On		