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ORIGINAL ARTICLE

Effect of wood, bark and leaf extracts of *Macaranga* trees on cytotoxic activity in some cancer and normal cell lines

Enos Tangke Arung[1] · Rudianto Amirta[2] · Qinchang Zhu[3,5] · Yhiya Amen[3,4] · Kuniyoshi Shimizu[3]

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Abstract The genus of *Macaranga* (Euphorbiacaceae) has 250 species of which 160 species are endemic in Kalimantan and New Guinea. They grow as pioneer trees and are used as traditional medicines in the Asian regions. This experiment concerns cytotoxicity in both cancer and normal cells of their methanol extracts from wood, bark and leaf parts. Some of them have not yet reported its cytotoxic activity in those cell lines. The 21 methanol extracts were prepared from seven *Macaranga* tree species (*Macaranga bancana*, *Macaranga gigantea*, *Macaranga hullettii*, *Macaranga pruinosa*, *Macaranga tanarius*, *Macaranga trichocarpa and Macaranga triloba*). The MTT assay was used to evaluate cytotoxic activity of extracts in cancer cell lines [human breast cancer (MCF-7), mouse melanoma (B16 melanoma), human colon cancer (HCT116), human cervical adenocarcinoma (HeLa)] and normal cell lines [human normal fibroblast (TIG-1) and normal human dermal fibroblast (NHDF)]. The leaf extracts of *M. pruinosa*, *M. tanarius*, *M. trichocarpa* showed more cytotoxicity than wood and bark in all cancer cell lines. In addition, the 5-fluorouracil was used as a positive control. These findings indicated the extracts from leaves of *M. pruinosa*, *M. tanarius* and *M. trichocarpa* had more potential than wood and bark parts to be developed as anticancer agents.

Keywords *Macaranga* trees · Cytotoxic activity · Cancer cells · Normal cells

Introduction

The genus of *Macaranga* (Euphorbiacaceae) has 250 species. About 30 species are distributed in tropical Africa and Madagascar, and the remaining are found in India, Sri Lanka, Myanmar, Indo-China, southern China, Taiwan, the Ryukyu Islands, Thailand, Malaysia region, northern Australia, the Pacific and Fiji. The Malaysian region is the main center of *Macaranga* diversity where about 160 species are located, and a very high number of endemics are also located in Borneo (Kalimantan) and New Guinea (Lemmens and Bunyapraphatsara 2003).

People in Asia traditionally use these trees in their daily lives. The traditional use of *M. bancana* in peninsular Malaysia includes using the leaves externally for treating boils, and in Sarawak, the powder of young leaves are applied as a paste to the skin to relive itch. The *M. gigantea* is used in peninsular Malaysia as decoction of the root bark to treat dysentery. In Sumatera, a decoction of the bark and leaves is used to treat stomachaches. The fresh sap is applied as an antidote to centipede bites. The *M. hullettii* is used in peninsular Malaysia as a decoction of leaves to

✉ Enos Tangke Arung
tangkearung@yahoo.com

✉ Kuniyoshi Shimizu
shimizu@agr.kyushu-u.ac.jp

[1] Laboratory of Forest Product Chemistry, Faculty of Forestry, Mulawarman University, Jl. KH Dewantara, Kampus Gn. Kelua, Samarinda, East Kalimantan 75123, Indonesia

[2] Laboratory of Industrial Biotechnology, Faculty of Forestry, Mulawarman University, Samarinda 75123, Indonesia

[3] Department of Agro-Environmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 819-0395, Japan

[4] Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

[5] Department of Pharmacy, School of Medicine, Shenzhen University, 3688 Nanhai Boulevard, Nanshan District, Shenzhen 518060, Guangdong, China

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treat stomachache, and wood is used as firewood. In the Philippines, the powder of the root of *M. tanarius* is used to treat fever, as well as a decoction of the root for hemoptysis. In the Moluccas and Papua New Guinea, the leaves are used to treat dysentery and an abortifacient. In peninsular Malaysia, pounded leaves are applied to wounds, and an infusion of the root is used to treat fever. The *M. triloba* in Java and Sumatera is used as a decoction of bark, leaves and fruits to treat stomachache (Lemmens and Bunyapraphatsara 2003). The young shoots of *M. gigantea*, *M. pruinosa and M. triloba* are used to treat fungal infections, while decoctions of their leaves are known to treat stomachaches (Grosvenor et al. 1995). Traditionally, there is no record of these trees used for cancer treatment.

A cancer cell is the uncontrolled proliferation and dedifferentiation of a normal cell. Cancer, a very serious problem in the human metabolic syndrome, is the major cause of mortality and morbidity all over the world. The number of cases is continuously rising. In the developed nations, cancer is the second major cause of death after cardiovascular disorders. The treatment of cancer involves surgery, radiotherapy and chemotherapy. In chemotherapy, the natural products have played a very important role for over 50 years (Singh et al. 2016; Kinghorn et al. 2016). Natural products from plants are a huge source of new chemical compounds and its derived compounds for chemotherapy candidates (Kinjo et al. 2016).

Based on traditional use for the medical treatment of Macaranga trees and abundant resources in East Kalimantan, in the present study, we focused on the medical function of these trees. We investigated the cytotoxicity effects of 23 methanol extracts prepared from 7 *Macaranga* tree species, named *Macaranga bancana*, *Macaranga gigantea*, *Macaranga hullettii*, *Macaranga pruinosa*, *Macaranga tanarius*, *Macaranga trichocarpa and Macaranga triloba* in order to find potential anti-cancer resources from these natural products.

Materials and methods

Chemicals

The dimethyl sulfoxide (DMSO) was purchased from Wako (Osaka, Japan). Ethylenediaminetetraacetic acid (EDTA) was from Dojindo Co, (Kumamoto, Japan). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were from Gibco (New York, USA). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Sigma (St. Louis, MO. USA), and Eagle's minimum essential medium (EMEM) was from Nissui Chemical Co (Osaka, Japan). Other chemicals used in this experiment were of the highest grade and commercially available.

Plant materials

The plants were collected in the Forest Education of Mulawarman University, Samarinda, East Kalimantan, Indonesia, in November, 2015. Voucher specimens were deposited in the Wood Chemistry Laboratory, Department of Forest Product Technology, Faculty of Forestry, Mulawarman University. The samples were identified by Raharjo, S.Hut (Laboratory of Dendrology, Forestry Faculty, Mulawarman University). A list of investigated species are shown in Table 1.

Preparation of plant extracts

Plant materials were dried at room temperature and powdered. The dried materials (each sample 50 g) were extracted with 360 ml of methanol at room temperature for 48 h. The extract solutions were filtered and concentrated in vacuo, to obtain the crude methanol extracts. The yields are shown in Table 1.

Cell culture

Normal human dermal fibroblast (NHDF) cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS). Human breast cancer (MCF-7) cells, human cervical adenocarcinoma (HeLa) cells and human normal fibroblast (TIG-1) cells were maintained in EMEM supplemented with 10% FBS. A mouse B16 melanoma cells were maintained in EMEM supplemented with 10% FBS and 0.09 mg/ml theophylline. Human colon cancer (HCT116) cells were maintained in McCoy's 5A medium supplemented with 10% FBS. All cancer cell lines and normal cell lines were obtained from RIKEN BioResource Center (Tsukuba, Ibaraki, Japan) and were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell viability

Cell viability was determined by using the microculture tetrazolium technique (MTT). The MTT assay provides a quantitative measure of the number of viable cells by determining the amount of formazan crystals produced by metabolic activity in treated versus controlled cells. In brief, confluent cells in 96-well plate or 24-well plate were treated with either vehicle or samples of different concentrations for 72 h and were then subjected to checks for the cell viability using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solutions. After a 4-h incubation period, the MTT solution was removed and

Table 1 Cell viability of different parts of some *Macaranga* species at 100 µg/ml in cancer and normal cell lines

Samples	Cell viability (%)[a]							
	Parts	Extract (g)	MCF-7	B16 melanoma	HCT116	HeLa	TIG-1	NHDF
Macaranga bancana	Bark	0.91	127.5 ± 0.06	105.3 ± 0.09	141.6 ± 0.02	60.8 ± 0.12	NT	NT
	Wood	0.74	99.6 ± 0.10	110.1 ± 0.14	132.1 ± 0.13	105.3 ± 0.07	NT	NT
	Leaf	7.05	131.3 ± 0.12	53.0 ± 0.14	75.6 ± 0.04	45.6 ± 0.09	NT	NT
Macaranga gigantea	Bark	0.33	103.2 ± 0.03	121.1 ± 0.02	92.2 ± 0.06	99.1 ± 0.07	NT	NT
	Wood	0.28	90.8 ± 0.11	94.6 ± 0.04	125.3 ± 0.06	114.1 ± 0.07	NT	NT
	Leaf	2.06	90.1 ± 0.07	50.0 ± 0.04	70.4 ± 0.03	46.5 ± 0.28	NT	NT
Macaranga hullettii	Bark	1.43	101.9 ± 0.11	121.4 ± 0.08	112.3 ± 0.08	116.0 ± 0.11	NT	NT
	Wood	0.68	124.6 ± 0.11	90.4 ± 0.01	81.1 ± 0.15	75.0 ± 0.05	NT	NT
	Leaf	6.00	137.8 ± 0.02	121.9 ± 0.18	122.2 ± 0.02	81.8 ± 0.39	NT	NT
Macaranga pruinosa	Bark	2.17	95.9 ± 0.02	148.6 ± 0.03	122.8 ± 0.13	94.7 ± 0.08	NT	NT
	Wood	0.48	96.4 ± 0.03	109.2 ± 0.02	149.3 ± 0.12	119.2 ± 0.13	NT	NT
	Leaf	6.40	34.8 ± 0.05	24.1 ± 0.01	27.3 ± 0.03	11.1 ± 0.03	35.1 ± 0.01	49.0 ± 0.01
Macaranga tanarius	Bark	1.06	95.5 ± 0.10	109.0 ± 0.24	77.3 ± 0.02	78.6 ± 0.31	NT	NT
	Wood	3.51	133.9 ± 0.05	106.0 ± 0.03	117.1 ± 0.02	104.7 ± 0.14	NT	NT
	Leaf	2.87	31.7 ± 0.01	11.1 ± 0.02	22.5 ± 0.01	15.7 ± 0.02	37.7 ± 0.01	51.1 ± 0.01
Macaranga trichocarpa	Bark	0.73	89.5 ± 0.03	143.6 ± 0.03	101.2 ± 0.05	86.3 ± 0.13	NT	NT
	Wood	0.57	128.5 ± 0.11	139.2 ± 0.01	112.1 ± 0.05	100.8 ± 0.06	NT	NT
	Leaf	5.40	48.4 ± 0.13	38.4 ± 0.01	28.4 ± 0.06	31.5 ± 0.31	44.3 ± 0.02	76.3 ± 0.03
Macaranga triloba	Bark	0.26	114.9 ± 0.05	100.4 ± 0.07	89.7 ± 0.05	76.9 ± 0.01	NT	NT
	Wood	0.37	115.6 ± 0.03	105.5 ± 0.08	168.8 ± 0.06	114.7 ± 0.07	NT	NT
	Leaf	6.20	119.4 ± 0.14	127.9 ± 0.08	102.4 ± 0.10	99.6 ± 0.21	NT	NT
5-FU			98.5 ± 0.05	24.3 ± 0.05	71.3 ± 0.02	94.3 ± 0.10	83.1 ± 0.01	94.9 ± 0.02

Data are represented as the mean ± SD (n = 3)

NT not tested

[a]% versus control

HCl-isopropanol solution was added to each well. The plate was incubated in the dark for 4 more hours, and the resulted solution was measured for absorbance at 570 nm with a microplate reader EL×800, Biotech (Winooski, Vermont, USA; Arung et al. 2010). Cell viability was calculated by the ratio of absorbance of sample-treated well to that of vehicle-treated well. The 50% inhibitory concentration (IC_{50}) was inferred from the viability-dose-dependent curve.

Results and discussion

Table 1 summarizes the scientific name, part used, cell viability of cancer cell lines and normal cell lines in concentration of 100 µg/ml. Leaf extracts of *M. pruinosa*, *M. tanarius* and *M. trichocarpa* showed < 50% of viability cell to cancer cell lines such as human breast cancer (MCF-7) cells, a mouse melanoma (B16 melanoma) cells, human colon cancer (HCT116) cells and human cervical adenocarcinoma (HeLa) cells. The viability cell of *M. pruinosa* and *M. tanarius* leaf extracts was almost similar with ranges of < 35% in MCF-7, < 25% in B16 melanoma, < 30% in HCT116 and < 20% in HeLa cells. The viability cell of *M. trichocarpa* was < 50% in MCF-7, < 40% in B16 melanoma, < 30% in HCT116 and < 35% in HeLa cells. The *M. bancana* and *M. gigantea* affected cell viability in B16 melanoma and HeLa with a range of ≤ 50%. Overall, the leaf extracts of *M. pruinosa*, *M. tanarius* and *M. trichocarpa* showed cytotoxicity effect on cancer cells. In contrast, leaves of *M. hulllettii* and *M. triloba* had no influence on any cancer cells. Most of bark and wood samples were less or had no affect on the cells.

In this experiment, *M. pruinosa*, *M. tanarius* and *M. trichocarpa* were depicted as the most potent cytotoxicity in the cells. Therefore, the extracts were tested on normal cells such as TIG-1 and NHDF cells for comparison. These extracts showed 35–45% cell viability of TIG-1 and 50–80% cell viability in NHDF cells. In addition, we

Table 2 The IC_{50} value of leaf extracts of *Macaranga* species in different cancer cell lines

Samples	IC_{50} (μg/ml)				
	Parts	MCF-7	B16 melanoma	HCT116	HeLa
Macaranga pruinosa	Leaf	460.8 ± 2.40	38.9 ± 0.28	70.48 ± 0.04	71.82 ± 1.05
Macaranga tanarius	Leaf	132.0 ± 3.70	13.26 ± 7.46	28.90 ± 0.06	19.57 ± 0.74
Macaranga trichocarpa	Leaf	187.0 ± 5.01	50.7 ± 0.23	56.64 ± 0.57	68.21 ± 0.22
5-FU[a]		98.5 ± 0.05	24.3 ± 0.05	71.3 ± 0.02	94.3 ± 0.10

Data are represented as the mean ± SD (n = 3)

[a]At 100 μl/ml

also compared the standard or positive control, named 5-fluorouracil (5-FU). The 5-FU had more of an effect on the B16 melanoma cell than any other cancer cells and less affect on normal cells.

The IC50 of *M. pruinosa*, *M. tanarius* and *M. trichocarpa* can be seen in Table 2. The leaf extract of *M. tanarius* was more potent than the others having a cytotoxicity effect in cancer cells namely 132.0 μg/ml in MCF-7, 13.26 μg/ml in B16 melanoma, 28.90 μg/ml in HCT116, 19.57 μg/ml in HeLa, respectively. The cytotoxicity effect of leaf extract from *M. pruinosa*, *M. tanarius* and *M. trichocarpa* may have been caused by some prenylated and geranylated flavonoids and also other compounds. Some prenylated and geranylated flavonoids (tanariflavanone C, tanariflavanone D and nymphaeol A) from leaves extract of *M. tanarius* depicted cytotoxicity in human oral carcinoma, human breast cancer and human small cell lung cancer (Phommart et al. 2005). Furthermore, Agustina et al. (2012) explained the cytotoxicity effect of leaf extract from *M. lowii* in mouse leukemia (P388) cells by macalowiinin, 4′-*O*-methyl-8-isoprenyl naringenin and acacetin. Some prenylated and geranylated flavonoids from twigs of *M. indica* named macarindicin A, macarindicin B, macarindicin C, broussoflavonol F, broussoflavonol G and macarangin observed cytotoxicity against cancer cells such as human breast adenocarcinoma (MCF-7), human hepatocellular (Hep G2), human cervical carcinoma (HeLa) and P388 cells (Yang et al. 2015a). Yang et al. (2015b) reported the prenylated and geranylated flavonoid such as 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one and 5,7,3′,4′-tetrahydroxy-6-geranylflavonol from fronds of *M. denticulata*-inhibited proliferation of human lung cancer cells (A549 cells). The twigs of *M. adenantha* revealed that some prenylated flavonoids (macadenanthin B and C) have cytotoxicity effects on cancer cell lines of MCF-7, Hep G2, HeLa and P388 (Yang et al. 2015c). The distribution of *Macaranga* genus (Euphorbiaceae) is wide in the tropical regions, and an abundant source of prenylated flavonoids and stilbenes, and the biological activities of those metabolites are very important for all fields of pharmacological sciences (Magadula 2014).

Conclusion

In summary, seven *Macaranga* trees from East Kalimantan, Indonesia, were investigated for its cytotoxicity effect in cancer cell lines (MCF-7, B16 melanoma, HCT116 and HeLa) and normal cell lines (TIG and NHDF). The leaf extracts of *M. pruinosa*, *M. tanarius* and *M. trichocarpa* showed the most potent cytotoxicity effect in four cancer cell lines compare with wood and bark. In addition, these extracts observed also cytotoxicity in normal cell lines such as TIG and NHDF. These findings indicate that *Macaranga* trees may be useful ingredients in anti-cancer agents, and further experiments such as fractionation of active fraction and isolation of active compounds and its mechanism are in progress.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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