Acta Pharm. **71** (2021) 153–162
https://doi.org/10.2478/acph-2021-0009

Short communication

In vitro antioxidant and antityrosinase activities of *Manilkara kauki*

SIRINADA SRISUPAP[1]
CHANYA CHAICHAROENPONG[2,3,*]

[1] *Program in Biotechnology, Faculty of Science, Chulalongkorn University Bangkok 10330, Thailand*

[2] *Institute of Biotechnology and Genetic Engineering, Chulalongkorn University Bangkok 10330, Thailand*

[3] *Molecular Crop Research Unit, Faculty of Science, Chulalongkorn University Bangkok 10330, Thailand*

Manilkara kauki L. Dubard is a tropical plant in the genus *Manilkara* of family Sapotaceae. This study investigated the total phenolic and flavonoid contents, and antioxidant and antityrosinase activities in different parts of *M. kauki* (fruits, leaves, seeds, stem barks and woods) and in fractions of stem barks. The total phenolic and flavonoid contents of the methanol and aqueous crude extracts of different parts of *M. kauki* ranged from 10.87 to 176.56 mg GAE (gallic acid equivalents) per gram of crude extract and 14.33 to 821.67 mg QE (quercetin equivalents) per gram of crude extract, resp. Leaves and stem barks exhibited higher total phenolic and flavonoid contents and antioxidant and antityrosinase activities than fruits, seeds and woods. Stem barks were sequentially extracted with *n*-hexane, ethyl acetate, methanol and water and then the fractionated extracts were subjected to antioxidant and antityrosinase activities testing. The ethyl acetate and methanol extracts of stem barks exhibited higher total phenolic and flavonoid contents and antioxidant and antityrosinase activities than the *n*-hexane and aqueous extracts. Moreover, ethyl acetate extract of *M. kauki* stem exhibited the highest antityrosinase activity. It may be a potential source of tyrosinase inhibitors for pharmaceutical and cosmetic applications.

Keywords: *Manilkara kauki*, total phenolic content, total flavonoid content, antioxidant, tyrosinase inhibitor

Accepted April 3, 2020
Published online April 21, 2020

In recent years, trends in using medicinal plants, such as phytotherapy, functional foods, herbal teas and especially cosmetics have been growing rapidly. Medicinal plants are commonly used as active ingredients of skin whitening products to treat hyperpigmentation. Melanin is essential for protecting human skin cells from ultraviolet radiation. However, overproduction of melanin leads to skin hyperpigmentation disorders such as blemishes, freckles, melasma, age spots and melanoma (1). Tyrosinase (EC 1.14.18.1) is a key enzyme in melanin biosynthesis. It catalyzes the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and the oxidation of L-DOPA to (2*S*)-2-amino-3-(3,4-dioxo-

* Correspondence; e-mail: chanya.c@chula.ac.th

cyclohexa-1,5-dien-1-yl)propanoic acid (DOPAquinone). DOPAquinone is spontaneously converted to 5,6-dioxo-2,3,5,6-tetrahydro-1*H*-indole-2-carboxylic acid (DOPAchrome). Then, DOPAchrome is transformed into 5,6-dihydroxyindole (DHI) and further oxidized and polymerized to melanin (2). Tyrosinase inhibitors prevent overproduction of melanin in the skin; kojic acid, α-arbutin, hydroquinone and vitamin C have been used as tyrosinase inhibitors in commercial skin whitening cosmetics (3). However, these tyrosinase inhibitors may have side-effects or risks causing skin allergies, inflammation, genotoxicity (4). Furthermore, some countries have banned kojic acid because of a potential connection to the development of cancer (5). Therefore, various phytochemicals have been investigated to be less toxic yet effective tyrosinase inhibitors. Several medicinal plants have been investigated and their *in vitro* and *in vivo* antityrosinase activities have been reported (6, 7).

Manilkara kauki (L.) Dubard is a tropical evergreen tree that belongs to the genus *Manilkara* of family *Sapotaceae*. The fresh ripe fruits are edible with a sweet taste and contain many nutrients such as proteins, fats, calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, vitamin C, thiamin, riboflavin and niacin (8). In traditional medicine in India, barks and roots of this plant are used as an astringent and to treat infantile diarrhea (9). Seeds are used as febrifuge, anthelmintic and antileprotic agents (9). Leaves are used as poultices for tumors (9). However, limited scientific studies of this plant have been found.

The aim of this study was to investigate the total phenolic and flavonoid contents as well as *in vitro* antioxidant and antityrosinase activities of crude extracts from various plant parts (fruits, leaves, seeds, stem barks and woods) of *M. kauki*.

EXPERIMENTAL

Chemicals and equipment

L-tyrosine, kojic acid, α-arbutin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid and quercetin were purchased from Merck (Germany). Mushroom tyrosinase, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), ferric chloride hexahydrate, aluminum chloride and Trolox were purchased from Sigma-Aldrich (USA).

A vacuum rotary evaporator (EYELA, Japan) and a microplate reader Multiscan GO (Thermo Fisher Scientific, USA) were used.

Plant materials

The fruits, leaves, seeds, stem barks and woods of *M. kauki* were collected from Bangkok, Thailand, in December 2015. The plants were identified by plant taxonomist in the Department of Botany, Faculty of Science, Chulalongkorn University, Thailand. The voucher specimens (BCU No. A015371) were deposited in the Professor Kasin Suvatabhandhu Herbarium, Chulalongkorn University, Thailand.

Preparation of plant parts extracts

Fresh fruits, leaves, seeds, stem barks and woods of *M. kauki* were dried in a hot air oven at 60 °C, then ground into powder. The ground samples (10 g each) were extracted

with methanol (70 mL × 3) for 72 h at room temperature or with water (70 mL × 3) for 20 minutes at 60 °C. Each extract was filtered through Whatman No. 1 filter paper and the filtrate was evaporated under reduced pressure to obtain methanol and aqueous crude extracts of five different parts of *M. kauki*.

In the case of stem barks the ground sample (500 g) was successively soaked in *n*-hexane (3 × 1 L), ethyl acetate (3 × 1 L) and methanol (3 × 1 L) for 72 h at room temperature and water (3 × 1 L) for 20 minutes at 60 °C. Each extract was filtered through Whatman No. 1 filter paper and the filtrate was evaporated under reduced pressure to obtain *n*-hexane, ethyl acetate, methanol and aqueous extracts of stem barks of *M. kauki*.

Determination of total phenolic content

The total phenolic content was determined using Folin-Ciocalteu reagent as previously described (10). The sample was dissolved in methanol (1 mg mL^{-1}). The assay was performed in triplicate for each analysis. Gallic acid (0–190 mg L^{-1}) was used as a standard phenolic compound. The total phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per gram of extract.

Determination of total flavonoid content

The total flavonoid content was measured using the aluminum chloride colorimetric assay (11). The sample was prepared in a concentration of 1 mg mL^{-1} in methanol. All analyses were carried out in triplicate. Quercetin (0–1000 mg L^{-1}) was used as a standard flavonoid. The total flavonoid content was expressed in milligrams of quercetin equivalents (QE) per gram of extract.

DPPH radical scavenging assay

DPPH assay was performed according to the previously reported method (12). The sample was dissolved in 95 % methanol (1 mg mL^{-1}). All analyses were performed in triplicate. The DPPH radical scavenging activity was expressed in the percentage of inhibition.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed by a modified spectrophotometric assay as previously reported (13). The sample was dissolved in 95 % methanol (1 mg mL^{-1}). The standard curve was linear between 25 and 1000 μg mL^{-1} of Trolox. The results were expressed in milligrams of Trolox equivalents (TE) per gram of extract.

Antityrosinase activity assay

The antityrosinase activity was measured by a slightly modified spectrophotometric method (14). L-tyrosine was used as a substrate and dissolved in 0.1 mol L^{-1} phosphate buffer (pH 6.5). Kojic acid and α-arbutin were used as positive controls and were also dissolved in 0.1 mol L^{-1} phosphate buffer (pH 6.5). The sample was prepared in 20 % dimethyl sulfoxide (DMSO) in ethanol. In the assay, 8 μL of the sample solution was mixed with 133 μL of 0.1

mol L^{-1} phosphate buffer (pH 6.5) and 47 μL of 2.5 mmol L^{-1} L-tyrosine. The reaction mixture was incubated for 5 min at room temperature. Then, 12 μL of mushroom tyrosinase solution (3224 U mL^{-1}) in 0.1 mol L^{-1} phosphate buffer (pH 6.5) was added to the reaction mixture. After incubation for 20 min at room temperature, the absorbance of the reaction mixture was recorded at 475 nm. The assay for each sample was performed in triplicate. The half-maximal inhibitory concentration (IC_{50}) values were calculated by linear regression analysis.

Statistical analysis

Statistical analyses were performed using a one-way analysis of variance (ANOVA) by SPSS program (version 17.0, SPSS Inc., USA). The significant difference between the means was determined by Duncan's multiple range test. $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Extraction and chemical composition of M. kauki *parts*

The results of the percentage yield of the methanol and aqueous crude extracts of different parts of *M. kauki* are shown in Table I.

Table I. Percentage yield of crude extracts of M. kauki *plant parts*

Plant part	Yield (%)	
	Methanol crude extract	Aqueous crude extract
Fruits	11.0	19.1
Leaves	11.6	9.0
Seeds	5.3	8.7
Stem barks	5.9	5.9
Woods	3.4	4.5

The total phenolic content in the methanol crude extract of five different parts of *M. kauki* ranged between 14.83 and 176.56 mg GAE g^{-1} crude extract whereas in aqueous crude extract it ranged 10.87 to 105.98 mg GAE g^{-1} crude extract (Table II).

Total flavonoid content in the methanol crude extract of different parts of *M. kauki*, ranged from 15.67 to 821.67 mg QE g^{-1} crude extract but in the aqueous crude extracts it was 14.33 to 800.67 mg QE g^{-1} crude extract (Table II).

Leaves and stem barks showed higher amounts of total phenolic and flavonoid contents than fruits, seeds and woods.

Table II. Total phenolic and flavonoid contents of M. kauki *plant parts*

Plant part	Total phenolic content (mg GAE g^{-1} crude extract)		Total flavonoid content (mg QE g^{-1} crude extract)	
	Methanol crude extract	Aqueous crude extract	Methanol crude extract	Aqueous crude extract
Fruits	17.52 ± 0.10[d]	14.83 ± 0.10[d]	99.00 ± 0.73[c]	105.67 ± 0.15[c]
Leaves	101.75 ± 1.63[b]	105.98 ± 0.10[a]	776.67 ± 2.08[b]	745.33 ± 2.52[b]
Seeds	14.83 ± 0.10[e]	10.87 ± 0.02[e]	38.33 ± 0.58[d]	43.33 ± 0.42[d]
Stem barks	176.56 ± 0.10[a]	99.73 ± 0.77[b]	821.67 ± 2.89[a]	800.67 ± 2.08[a]
Woods	83.68 ± 0.87[c]	47.71 ± 0.67[c]	15.67 ± 0.31[e]	14.33 ± 0.25[e]

Data are presented as mean ± SD of triplicates.
[a-e] Values with different superscript letters within the same column are significantly different ($p < 0.05$).

Antioxidant activity of M. kauki *parts*

DPPH radical scavenging activity. – The results of DPPH radical scavenging activity testing of different parts of *M. kauki* are presented in Table III. Concerning the methanol crude extracts of five different parts of this plant, stem barks exhibited the highest scavenging DPPH capacity (57.9 ± 0.3 %), followed by leaves (47.0 ± 0.1 %), woods (43.5 ± 0.3 %), fruits (8.8 ± 0.2 %) and seeds (2.2 ± 0.1 %), resp., at a concentration of 1 mg mL^{-1}. For the aqueous crude extracts, the order of DPPH activity was as follows: leaves (55.5 ± 0.1 %), stem barks (48.9 ± 0.1 %), woods (14.8 ± 0.1 %), fruits (1.0 ± 0.1 %) and seeds (0.4 ± 0.03 %), resp., at a concentration of 1 mg mL^{-1}.

FRAP activity. – As shown in Table III, the methanolic crude extracts of leaves (209.74 ± 1.28 mg TE g^{-1} crude extract) and stem barks (211.59 ± 1.40 mg TE g^{-1} crude extract) exhibited strong FRAP activity but the aqueous crude extract of leaves (219.37 ± 0.32 mg TE g^{-1} crude extract) showed the highest FRAP activity. Methanol and aqueous crude extracts of fruits, seeds and woods showed weak FRAP activity.

Table III. DPPH radical scavenging and FRAP activities of M. kauki *plant parts*

Plant part	DPPH radical scavenging activity (%)		FRAP activity (mg TE g^{-1} crude extract)	
	Methanol crude extract	Aqueous crude extract	Methanol crude extract	Aqueous crude extract
Fruits	8.80 ± 0.16[d]	1.03 ± 0.05[d]	11.96 ± 0.64[d]	5.48 ± 0.32[d]
Leaves	47.01 ± 0.14[b]	55.49 ± 0.06[a]	209.74 ± 1.28[a]	219.37 ± 0.32[a]
Seeds	2.21 ± 0.05[e]	0.41 ± 0.03[e]	16.41 ± 0.85[c]	4.19 ± 0.32[d]
Stem barks	57.87 ± 0.27[a]	48.89 ± 0.14[b]	211.59 ± 1.40[a]	107.33 ± 0.96[b]
Woods	43.51 ± 0.27[c]	14.75 ± 0.06[c]	72.70 ± 0.85[b]	41.59 ± 0.85[c]

Data are presented as mean ± SD of triplicates.
[a-e] Values with different superscript letters within the same column are significantly different ($p < 0.05$).

Antityrosinase activity of M. kauki *parts*

Among the methanolic crude extracts, stem barks exhibited the strongest antityrosinase activity with an IC_{50} value of 0.25 ± 0.03 mg mL^{-1}, followed by leaves, fruits and seeds, resp. For the aqueous crude extracts, the order of antityrosinase activity was as follows: stem barks, leaves, fruits and seeds, resp., whereas, the methanol and aqueous crude extracts of woods showed no antityrosinase activity (Table IV). Furthermore, the IC_{50} values for mushroom tyrosinase inhibitory activity of α-arbutin and kojic acid were 7.05 ± 0.22 mmol L^{-1} and 57.49 ± 0.35 μmol L^{-1}, resp.

Table IV. Antityrosinase activity of M. kauki *parts*

Plant part	IC_{50} (mg mL^{-1})	
	Methanol crude extract	Aqueous crude extract
Fruits	0.63 ± 0.01^{c}	0.86 ± 0.03^{c}
Leaves	0.38 ± 0.01^{b}	0.50 ± 0.01^{b}
Seeds	5.56 ± 0.03^{d}	6.73 ± 0.03^{d}
Stem barks	0.25 ± 0.03^{a}	0.41 ± 0.01^{a}
Woods	No activity	No activity

Data are presented as mean ± SD of triplicates.
$^{a-d}$ Values with different superscript letters within the same column are significantly different ($p < 0.05$).

The *Manilkara* spp. have been reported to possess pharmacological activities such as antioxidant, antimicrobial, anticancer, antidiabetic, antiulcer, analgesic, antityrosinase and immunomodulatory (15–19). The secondary metabolites of *Manilkara* spp. have also been reported to consist of flavonoids, phenolic compounds, tannins, triterpenes and saponins (18–21). Phenolic compounds and flavonoids are secondary metabolites of plants that have been shown to play an important role in antioxidant, antityrosinase, anticancer and other activities (22, 23). The inhibition of tyrosinase activity also depends on the hydroxyl group of the phenolic compounds. The hydroxyl group can develop a hydrogen bond at the active site of tyrosinase, resulting in steric hindrance or conformation changing and then leading to a lower enzymatic activity (24).

In the present study, both the methanol and aqueous crude extracts of leaves and stem barks showed strong DPPH radical scavenging, FRAP and antityrosinase activities, which might be ascribed to the presence of phenolic compounds and flavonoids. Similarly, previous studies of *Manilkara* spp. have reported a correlation between the antioxidant and antityrosinase activities and the amount of phytochemicals, including phenolic compounds and flavonoids (25). Some studies have reported that the plant extracts which contain high total phenolics or flavonoid contents exhibit strong antioxidant activity (15). Expectedly, the plant extracts with high amounts of phenolic compounds also possess high amounts of flavonoids (26, 27). In this study, stem barks of *M. kauki* displayed the highest *in vitro* antityrosinase activity compared with fruits, leaves, seeds and woods. The attention was, therefore, focused on stem barks. Hence, different polarities of solvents (*n*-hexane,

ethyl acetate, methanol and water) were used for the extraction of active substances of diverse lipophilicities. These fractions were evaluated for phytochemical composition (phenolic compounds and flavonoids), antioxidant and antityrosinase activities.

Extraction and chemical composition of M. kauki *stem barks*

The extraction yield of *n*-hexane, ethyl acetate, methanol and aqueous extracts of *M. kauki* stem barks were 3.3, 1.5, 16.9 and 9.8 %, respectively.

The total phenolic and flavonoid contents of the extracts of stem barks are shown in Table V. The methanol extract (160.12 ± 0.93 mg GAE g^{-1} extract) exhibited the highest total phenolic content, followed by ethyl acetate, aqueous and *n*-hexane extracts, resp. In addition, the ethyl acetate extract (755.00 ± 1.00 mg QE g^{-1} extract) showed the highest total flavonoid content, followed by methanol, aqueous and *n*-hexane extracts, resp.

Table V. Total phenolic and flavonoid contents of M. kauki *stem barks*

Fraction extract	Total phenolic content (mg GAE g^{-1} extract)	Total flavonoid content (mg QE g^{-1} extract)
n-Hexane	10.98 ± 0.10[d]	283.67 ± 1.15[d]
Ethyl acetate	111.46 ± 0.02[b]	755.00 ± 1.00[a]
Methanol	160.12 ± 0.93[a]	527.33 ± 0.58[b]
Aqueous	66.17 ± 0.48[c]	316.67 ± 1.15[c]

Data are presented as mean ± SD of triplicates.
[a-d] Values with different superscript letters within the same column are significantly different ($p < 0.05$).

Antioxidant activity of M. kauki *stem barks*

DPPH radical scavenging activity. – The ethyl acetate and methanol extracts exhibited high DPPH radical scavenging activity with 55.7 ± 0.1 % and 54.2 ± 0.2 %, resp., at a concentration of 1 mg mL^{-1}, whereas *n*-hexane and aqueous extracts showed weak DPPH radical scavenging activity (Table VI).

Table VI. Antioxidant and antityrosinase activities of M. kauki *stem barks*

Extract	Antioxidant activities		Antityrosinase activity IC_{50} (mg mL^{-1})
	DPPH radical scavenging activity (%)	FRAP activity (mg TE g^{-1} extract)	
n-Hexane	0.66 ± 0.03[d]	10.11 ± 0.56[d]	9.58 ± 0.10[d]
Ethyl acetate	55.69 ± 0.13[a]	180.83 ± 0.81[b]	0.24 ± 0.04[a]
Methanol	54.22 ± 0.17[b]	221.59 ± 0.32[a]	1.62 ± 0.01[b]
Aqueous	7.24 ± 0.05[c]	50.85 ± 0.32[c]	1.91 ± 0.02[c]

Data are presented as mean ± SD of triplicates.
[a-d] Values with different superscript letters within the same column are significantly different ($p < 0.05$).

FRAP activity. – The ethyl acetate and methanol extracts also exhibited potent FRAP activity (180.83 ± 0.81 and 221.59 ± 0.32 mg TE g^{-1} extract, resp.), while, the *n*-hexane and aqueous extracts exhibited weak FRAP activity (Table VI).

Antityrosinase activity of M. kauki *stem barks*

The ethyl acetate extract (IC_{50} = 0.24 ± 0.04 mg mL^{-1}) exhibited the strongest antityrosinase activity, followed by methanol (IC_{50} = 1.62 ± 0.01 mg mL^{-1}), aqueous (IC_{50} = 1.91 ± 0.02 mg mL^{-1}) and *n*-hexane (IC_{50} = 9.58 ± 0.10 mg mL^{-1}) extract.

The partial purification of *M. kauki* stem barks indicated that high amounts of phenolic compounds and flavonoids were found in the ethyl acetate and methanol extracts as a medium to high polarity media. These extracts also demonstrated high antioxidant and antityrosinase activities. According to the reports on some other plants such as *Zingiber officinale*, *Armoracia rusticana*, *Bucida buceras* and *Phoradendron californicum*, the amounts of phenolic compounds and flavonoids, antioxidant and antityrosinase capacities also differed depending on the polarity of solvents for extraction (28–30).

CONCLUSIONS

This study was the first report on comparing phytochemical composition, antioxidant and antityrosinase activities of different parts of *M. kauki*. Its leaves and stem barks exhibited higher total phenolic and flavonoid contents, DPPH radical scavenging, FRAP and antityrosinase activities than fruits, seeds and woods. Furthermore, extraction with solvents of different polarities indicated that the ethyl acetate and methanol extracts of stem barks demonstrated high total phenolic and flavonoid contents, and antioxidant activity. However, only the ethyl acetate extract exhibited potent antityrosinase activity and may serve as a source of tyrosinase inhibitor for pharmaceutical and cosmetic applications.

We are inclined to believe that a high level of phenolic compounds/flavonoids led to good antioxidant and antityrosinase activities. This comes along with the fact that ethyl acetate extract of stem barks with a significantly higher amount of flavonoids exhibited also significantly higher antityrosinase activity, pointing to the fact that flavonoids play an important role in tyrosinase inhibition.

Acknowledgements. – This work was supported by the Graduate School of Chulalongkorn University (thesis grant and conference grant for master degree student) and the Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (GRU 6203023003-1).

REFERENCES

1. J. L. Rees, The genetics of human pigmentary disorders, *J. Invest. Dermatol*. **131** (2011) E12-E13; https://doi.org/10.1038/skinbio.2011.5
2. V. J. Hearing, Determination of melanin synthetic pathways, *J. Invest. Dermatol*. **131** (2011) E8-E11; https://doi.org/10.1038/skinbio.2011.4
3. C. Couteau and L. Coiffard, Overview of skin whitening agents: drugs and cosmetic products, *Cosmetics* **3** (2016) 27–43; https://doi.org/10.3390/cosmetics3030027

4. J. F. Hsieh, S. T. Chen and S. L. Cheng, *Molecular Profiling of A375 Human Malignant Melanoma Cells Treated with Kojic Acid and Arbutin, Breakthroughs in Melanoma Research*, in *Breakthroughs in Melanoma Research* (Ed. Y. Tanaka), InTech, Shanghai 2011, pp. 533–558.
5. S. L. Cheng, R. H. Liu, J. N. Sheu, S. T. Chen, S. Sinchaikul and G. J. Tsay, Toxicogenomics of kojic acid on gene expression profiling of A375 human malignant melanoma cells, *Biol. Pharm. Bull*. **29** (2006) 655–669; https://doi.org/10.1248/bpb.29.655
6. T. Pillaiyar, V. Namasivayam, M. Manickam and S. H. Jung, Inhibitors of melanogenesis: an updated review, *J. Med. Chem*. **61** (2018) 7395–7418; https://doi.org/10.1021/acs.jmedchem.7b00967
7. P. K. Mukherjee, R. Biswas, A. Sharma, S. Banerjee, S. Biswas and C. K. Katiyar, Validation of medicinal herbs for anti-tyrosinase potential, *J. Herb. Med*. **14** (2018) 1–16; https://doi.org/10.1016/j.hermed.2018.09.002
8. T. K. Lim, *Edible Medicinal and Non-Medicinal Plants, Vol. 6. Fruits*, Springer, Dordrecht 2013, pp. 107–109.
9. C. P. Khare, *Indian Medicinal Plants: An Illustrated Dictionary*, Springer Verlag, New York 2007, pp. 397–398.
10. E. A. Ainsworth and K. M. Gillespie, Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent, *Nat. Protoc*. **2** (2007) 875–877; https://doi.org/10.1038/nprot.2007.102
11. B. Tohidi, M. Rahimmalek and A. Arzani, Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran, *Food Chem*. **220** (2017) 153–161; https://doi.org/10.1016/j.foodchem.2016.09.203
12. K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D. H. Byrne, Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, *J. Food Comp. Anal*. **19** (2006) 669–675; https://doi.org/10.1016/j.jfca.2006.01.003
13. I. F. F. Benzie and J. J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": The FRAP assay, *Anal. Biochem*. **239** (1996) 70–76; https://doi.org/10.1006/abio.1996.0292
14. A. Ishihara, Y. Ide, T. Bito, N. Ube, N. Endo, K. Sotome, N. Maekawa, K. Ueno and A. Nakagiri, Novel tyrosinase inhibitors from liquid culture of *Neolentinus lepideus*, *Biosci. Biotechnol. Biochem*. **82** (2018) 22–30; https://doi.org/10.1080/09168451.2017.1415125
15. V. Kothari, S. Pathan and S. Seshadri, Antioxidant activity of *Manilkara zapota* and *Citrus limon* seeds, *J. Nat. Remedies* **10** (2010) 175–180; https://doi.org/10.18311/jnr/2010/259
16. M. A. Osman, M. A. Aziz, M. R. Habib and M. R. Karim, Antimicrobial investigation on *Manilkara zapota* (L.) P. Royen, *Int. J. Drug Dev. Res*. 3 (2011) 185–190.
17. K. J. Pankaj, S. Prashant, U. Neeraj and S. Yogesh, Evaluation of analgesic activity of *Manilkara zapota* (leaves), *Eur. J. Exp. Biol*. 1 (2011) 14–17.
18. N. M. Fayek, A. R. A. Monem, M. Y. Mossa, M. R. Meselhy and A. H. Shazly, Chemical and biological study of *Manilkara zapota* (L.) Van Royen leaves (Sapotaceae) cultivated in Egypt, *Pharmacogn. Res*. **4** (2012) 85–91; https://doi.org/10.4103/0974-8490.94723
19. N. M. Fayek, A. R. A. Monem, M. Y. Mossa and M. R. Meselhy, New triterpenoid acyl derivatives and biological study of *Manilkara zapota* (L.) Van Royen fruits, *Pharmacogn. Res*. **5** (2013) 55–59; https://doi.org/10.4103/0974-8490.110505
20. F. B. de Almeida, C. P. Fernandes, W. Romao, G. Vanini, H. B. Costa, H. S. Franca, M. G. Santos, J. C. T. Carvalho, D. Q. Falcao and L. Rocha, Secondary metabolites from leaves of *Manilkara subsericea* (Mart.) Dubard, *Pharmacogn. Mag*. **11** (2015) S533–S537; https://doi.org/10.4103/0973-1296.172957
21. M. H. Baky, A. M. Kamal, M. R. Elgindi and E. G. Haggag, A review on phenolic compounds from family Sapotaceae, *J. Pharmacogn. Phytochem*. **5** (2016) 280–287.

22. A. Ghasemzadeh and N. Ghasemzadeh, Flavonoids and phenolic acids: role and biochemical activity in plants and human, *J. Med. Plants Res.* **5** (2011) 6697–6703; https://doi.org/10.5897/JMPR11.1404
23. S. Kumar and A. K. Pandey, Chemistry and biological activities of flavonoids: an overview, *Sci. World J.* **2013** (2013) 162750; https://doi.org/10.1155/2013/162750
24. H. S. Baek, H. S. Rho, J. W. Yoo, S. M. Ahn, J. Lee, M. K. Kim, D. H. Kim and I. S. Chang, The inhibitory effect of new hydroxamic acid derivatives on melanogenesis, *Bull. Korean Chem. Soc.* **29** (2008) 43–46; https://doi.org/10.5012/bkcs.2008.29.1.043
25. I. Corradi, E. de Souza, D. Sande and J. A. Takahashi, Correlation between phenolic compounds contents, antityrosinase and antioxidant activities of plant extracts, *Chem. Eng. Trans.* **64** (2018) 109–114; https://doi.org/10.3303/CET1864019
26. G. S. Jimenez, C. R. Aquino, L. C. Martinez, K. B. Torres and M. R. Monroy, Antioxidant activity and content of phenolic compounds and flavonoids from *Justicia spicigera*, *J. Biol. Sci.* **9** (2009) 629–632; https://doi.org/10.3923/jbs.2009.629.632
27. A. A. Elzaawely and S. Tawata, Antioxidant activity of phenolic rich fraction obtained from *Convolvulus arvensis* L. leaves grown in Egypt, *Asian J. Crop. Sci.* **4** (2012) 32–40; https://doi.org/10.3923/ajcs.2012.32.40
28. A. Ghasemzadeh, H. Z. E. Jaafar and A. Rahmat, Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts, *J. Med. Plants Res.* **5** (2011) 1147–1154.
29. L. Tomsone, Z. Kruma and R. Galoburda, Comparison of different solvents and extraction methods for isolation of phenolic compounds from horseradish roots (*Armoracia rusticana*), *Int. Sch. Sci. Res. Innov.* **6** (2012) 236–241; https://doi.org/10.5281/zenodo.1071162
30. S. B. Iloki-Assanga, L. M. Lewis-Luján, C. L. Lara-Espinoza, A. A. Gil-Salido, D. Fernandez-Angulo, J. L. Rubio-Pino and D. D. Haines, Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*, *BMC Res. Notes* **8** (2015) 396–409; https://doi.org/10.1186/s13104-015-1388-1