

Isolation of Carbazole Alkaloids and Coumarins from *Aegle marmelos* and *Murraya koenigii* and Their Antioxidant Properties

(Pengasingan Alkaloid dan Koumarin Karbazol daripada *Aegle marmelos* dan *Murraya koenigii* serta Sifat Antioksidannya)

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ABSTRACT

In this paper, antioxidant properties of Aegle marmelos (stem bark, leaves) and Murraya koenigii (stem bark, root) were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, 2,2'-Azino-bis(3-ethylbenzthiozoline-6-sulfonic acid) (ABTS) decolourisation, cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP) and linoleic acid/ β -carotene assays. The chloroform extract of Murraya koenigii stem bark was found to possess the highest antioxidant activity in CUPRAC (1490.89 mgTE/g extract). In contrary, the hexane extract from Aegle marmelos leaves exhibited the weakest antioxidant activity in the DPPH assay (81.06 mgTE/g extract). The bioactive compound mahanimbine (7) isolated from the stem bark of Murraya koenigii was found to be the most active antioxidant agent with TEAC of 927.73 and 1649.31 mgTE/g corresponding to the ABTS and CUPRAC assays, respectively, as well as a good lipid peroxidation inhibitor with an inhibitory percentage of 70.95%. These CUPRAC and ABTS assays are the first report for Malaysian Murraya koenigii species.

Keywords: *Aegle marmelos*; antioxidant; CUPRAC; mahanimbine; *Murraya koenigii*

ABSTRAK

Dalam kertas ini, sifat antipengoksida Aegle marmelos (kulit batang, daun) dan Murraya koenigii (kulit batang, akar) dinilai oleh perencatan radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH), Asid-2,2'-azino-bis(3-etilbenzthiozolin-6-sulfonik) (ABTS), kapasiti antipengoksida penurunan ion kuprik (CUPRAC), kuasa antioksidan penurunan ion ferik (FRAP) dan asid linoleat/ β -karotena. Ekstrak kloroform kulit batang Murraya koenigii didapati memiliki aktiviti antioksidan tertinggi dalam CUPRAC (1490.89 mgTE/g ekstrak). Sebaliknya, ekstrak heksana daripada daun Aegle marmelos memperlihatkan aktiviti antioksidan paling lemah dalam penilaian DPPH (81.06 mgTE/g ekstrak). Mahanimbine (7) merupakan sebatian yang diasingkan daripada kulit batang Murraya koenigii didapati sebagai agen antioksidan paling aktif dengan TEAC daripada 927.73 mgTE/g sampel dan 1649.31 mgTE/g sampel sepadan dengan ujian ABTS dan CUPRAC serta perencat lipid yang baik dengan peratusan perencatan 70.95%. Ujian CUPRAC dan ABTS ini merupakan laporan pertama untuk spesies Malaysia Murraya koenigii.

Kata kunci: *Aegle marmelos*; antioksidan; CUPRAC; mahanimbin; *Murraya koenigii*

INTRODUCTION

The Rutaceae are small trees, shrubs and herbs mainly found in tropical and sub-tropical region (Zhang et al. 2008). This family, also known as the rue or orange family includes citrus, *Murraya*, *Aegle*, *Melicope* and *Micromelum*. *Aegle marmelos* and *Murraya koenigii* have been used in traditional medicine to treat cancer, diabetes, inflammation and as an anti-microbial agent. Biological studies on this genus showed cytotoxic, anti-microbial and antioxidant activities. Based on the applications of these plants in traditional medicines, phytochemical studies were carried out and indicated the genus to be rich with interesting bioactive compounds such as alkaloids, coumarins, flavonoids and some plant steroids (Özkan et al. 2013).

Bioactive compounds are implemented progressively in a wide range of applications such as geomedicine, in plant science, modern pharmacology, agrochemical and food industry. The presence of bioactive compounds in plants and certain food such as vegetables, nuts, fruits, oils and wholegrain are good for maintenance of health (Guaadaou et al. 2014). Bernhoft (2010) defined plant bioactive compounds as secondary plant metabolites which induce pharmacological and toxicological effects in human and animals. The definition can be extended from a food to an isolated pure compound that could be ingested in a higher amount than those obtained through diet. They may exert drug-like properties with more potent efficacies and lower risks (Weaver 2014). Research associated with natural antioxidants has become popular in food chemistry,

food biology, natural plant chemistry, medicinal plants and biochemistry (Moon & Shibamoto 2009). Many epidemiological studies suggest that the consumption of nutritional food such as vegetable, fruits, polyphenol rich food or teas prevent degenerative diseases associated with aging (Almeida et al. 2011).

The acetone extract of *Murraya koenigii* showed the strongest antioxidant activity compared to the ethanol and methanol extracts (Singh et al. 2011). A previous study on *Murraya koenigii* showed this plant to be a potential antioxidant, chemopreventive and antimicrobial agent (Jagan Mohan Rao et al. 2007; Ningappa et al. 2008; Sasidharan & Menon 2010). The biological properties of *Aegle marmelos* were widely studied in Thailand and India. The antioxidant capabilities were studied on its essential oils through DPPH, FRAP, TPC, ABTS and ion chelation activity assays in which the results proposed *Aegle marmelos* to be a good antioxidant agent (Ariharan & Prasad 2014; Satyal et al. 2012). However, a study on the Indonesian *Aegle marmelos* species is rarely reported especially the hexane, chloroform and ethyl acetate extracts from this species have not been reported as most of the researches were focused on the alcoholic and aqueous extracts.

In continuation of our study on phytonutrients of *Rutaceae* family, the Indonesian *Aegle marmelos* and Malaysian *Murraya koenigii* were phytochemically and biologically studied for their antioxidant capacities. In this paper, the nutritional values of *Aegle marmelos* and *Murraya koenigii* were measured using various antioxidant assays and the bioactive compounds from the plants isolated and identified.

MATERIALS AND METHODS

PLANT MATERIALS

Leaves and stem bark of *Aegle marmelos* were collected from Jogjakarta, Indonesia and the specimen voucher was deposited at Herbarium of Faculty of Pharmacy, Gadjah Mada University, Indonesia. Meanwhile, *Murraya koenigii* plant was collected from Sungai Buloh, Selangor. The voucher specimen of *Murraya koenigii* (H009) was deposited at the Faculty of Forestry, Universiti Putra Malaysia.

PREPARATION OF EXTRACTS

The collected plant parts were extracted successively with hexane, chloroform and ethyl acetate. The plant samples were extracted for 72 h and repeated twice for the same solvent. The extraction was filtered the filtrates for the same solvent were pooled and concentrated using rotatory evaporator. The extracts of the leaves of *Aegle marmelos* (1.7 kg) were filtered and concentrated to give hexane (2.05 g), chloroform (28.98 g) and ethyl acetate (13.52 g) extracts, respectively. Meanwhile, the stem bark (1.3 kg) of *Aegle marmelos* afforded 4.40 g of hexane extract, 6.21

g chloroform extracts and 3.35g ethyl acetate extract. The extraction of the roots of *Murraya koenigii* (0.7 kg) yielded hexane, chloroform and ethyl acetate extracts with masses of 18.38, 14.95 and 6.88 g, respectively. Lastly, the stem bark of *Murraya koenigii* (1.1 kg) was concentrated to give hexane, chloroform, and ethyl acetate extracts with masses of 17.30, 12.13 and 5.28 g, respectively.

ISOLATION OF BIOACTIVE CONSTITUENTS

The extracts were chromatographed by gravity Column Chromatography prepared by silica 60 (70-230 mesh ASTM), MERCK 7734 and silica gel (20-400 mesh ASTM) MERCK 9385. The profiles of each fraction were monitored by using Thin Layer Chromatography (TLC), aluminium sheet precoated with silica gel 60 F₂₅₄ (20 × 20 cm). The potential fractions were further chromatographed and purified to obtain pure compounds.

The fractionation of the extracts from *Aegle marmelos* afforded 2 coumarins: marmin (**1**, 39.9 mg), 7-hydroxycoumarin (**2**, 24.2 mg); one alkaloid: aegeline (**3**, 10.0 mg), and 2 triterpenoids: epi-lupeol (**4**, 6.5 mg) and stigmaterol (**5**, 5.0 mg). Meanwhile, the isolation on the roots and stem bark of *Murraya koenigii* gave 4 alkaloids namely girinimbine (**6**, 328.5 mg), mahanimbine (**7**, 23.2 mg), murrayanine (**8**, 8.5 mg) and murrayacine (**9**, 5.1 mg) along with one triterpenoid: stigmaterol (**5**, 8.0 mg). Structural elucidations of the pure isolated compounds were achieved with the aid of various spectroscopic methods including nuclear magnetic resonance, mass spectroscopy, infrared and melting point. All the spectroscopic data were compared with literature data. The structures of the chemical constituents isolated from the *Rutaceae* species are shown in Figure 1.

IN-VITRO ANTIOXIDANT ASSAYS

The antioxidant capacities of the extracts and the isolated compounds were evaluated on their free radical scavenging ability through DPPH and ABTS assays, whereas ion reducing power was investigated via CUPRAC and FRAP assays. All samples were prepared in the concentration of 1 mg/mL and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as the standard in all the assays. A calibration curve of Trolox was set up and the results were expressed as Trolox Equivalence Antioxidant Capacity (TEAC) in the unit of mgTE/g sample. The absorbances of the samples were obtained via SPECTROstar Nano Microplate reader (BMG LABTECH, Offenburg, Germany).

DPPH FREE RADICAL SCAVENGING

The determination of DPPH scavenging activity was carried out following the method of Yeap et al. (2017) with slight modifications. Briefly, DPPH stock solution was prepared by dissolving 40 mg DPPH in 100 mL methanol. The prepared stock solution was further diluted with methanol to obtain an absorbance of 1.0 ± 0.01 unit at 517 nm wavelength using a microplate reader. 50 µL of sample

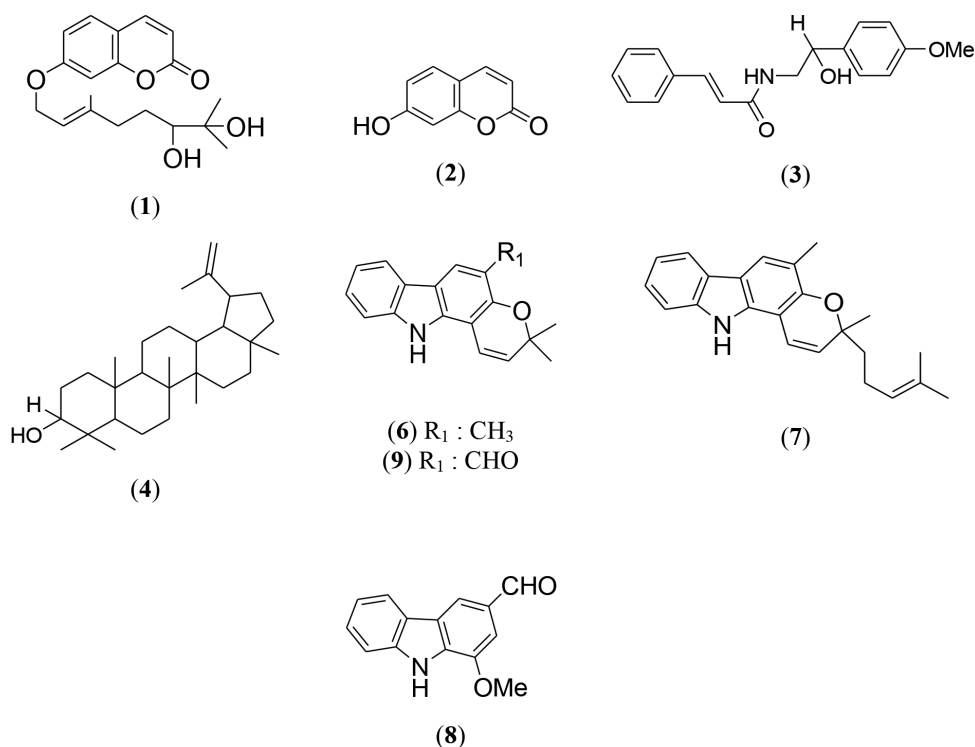


FIGURE 1. Chemical constituents isolated from *Aegle marmelos* and *Murraya koenigii*: Marmin (1), 7-hydroxycoumarin (2), aegeline (3), epi-lupeol (4), girinimbine (6), mahanimbine (7), murrayanine (8) and murrayacine (9)

was mixed with 1 mL methanolic DPPH solution and was kept for scavenging reaction in the dark for 30 min. The scavenging activity was read at wavelength 517 nm in a spectrophotometer after 30 min.

ABTS RADICAL CATION DECOLOURISATION

The procedure was adapted from the method of Yeap et al. (2017) with some modifications. In brief, the stock solution of 7.4 mM ABTS^{•+} and 2.6 mM of potassium persulfate was prepared. The working solution was prepared by mixing the two stock solutions in equal amounts and incubated for 12 h at room temperature in the dark. The mixture of stock solution was diluted in ethanol to be used as the working reagent. 1 mL of the working reagent was added into 50 μ L of test sample in the 96 well microplate and incubated in the dark. The reading was taken at absorbance 734 nm using a spectrophotometer.

COPPER REDUCING ANTIOXIDANT CAPABILITY (CUPRAC)

The determination of antioxidant activity by CUPRAC was carried out by adapting the method of Yeap et al. (2017). The working solutions were prepared by mixing 10 mM of Cu(II), 7.5 mM neocuprine, 1 M of ammonium acetate buffer and distilled water. 50 μ L of test samples were added into the 96 well microplate followed by addition of 1 mL of working solution. The plate was then incubated for 30 min. The microplate was read at 450 nm wavelength in a spectrophotometer after 30 min.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The determination of antioxidant activities through FRAP was carried out according to the method of Yeap et al. (2017). FRAP reagents were prepared fresh using 300 mM acetate buffer, pH 3.6; 10 mM TPTZ, in 40 mM HCL; and 20 mM FeCl₃•6H₂O in a ratio of 10:1:1 to give the working solution. 1 mL FRAP working solution was added into 50 μ L test samples in a 96 well microplate and the measurement was taken at 595 nm wavelength in a spectrophotometer after 30 min.

LINOLEIC ACID / B-CAROTENE BLEACHING ASSAY

The β -carotene bleaching assay employed is as previously described (Kassim et al. 2013) with slight modifications. In brief, 210 μ L of β -carotene solution was added into a round bottom flask containing 5 μ L of linoleic acid and 42 μ L of Tween 20. The chloroform from the mixture solution was removed by rotary evaporator. 10 mL of distilled water was added into the mixture and shaken vigorously to form an emulsion. 200 μ L of aliquot emulsion was added into 50 μ L test samples in the 96 well microplate and the measurement was taken at 470 nm wavelength using a spectrophotometer before and after incubation (t = 0 min and 2 h) in the incubator at 50°C. The antioxidant activity (AA) was calculated according to the following formula:

$$AA \% = 1 - [(A_{t=0} - A_{t=2}) / (A_{C=0} - A_{C=2})] \times 100$$

where $A_{t=0}$ and $A_{t=2}$ is the absorbance of the test samples measured at 0 and 2 h, respectively; and $A_{C=0}$ and $A_{C=2}$ is the absorbance of control measured at 0 and 2 h, respectively.

STATISTICAL ANALYSIS

All the experiments were performed in duplicate and repeated thrice independently and the results were expressed as mean \pm SD (standard deviation) and analyzed by the One-way ANOVA and followed by a Tukey's multiple comparison tests. Statistical analysis was performed using GraphPad Prism 7 and Microsoft Excel 2010.

RESULTS AND DISCUSSION

ANTIOXIDANT CAPACITIES OF *AEGLE MARMELLOS* AS MEASURED BY DPPH, ABTS, FRAP AND CUPRAC ASSAYS

Figure 2 shows the antioxidant activities of the stem bark and leaf extracts of *Aegle marmelos*. The leaves and stem bark extracts from *Aegle marmelos* gave the highest activity measured by CUPRAC except hexane extract from stem bark which exhibited highest activity in DPPH assays. Based on the results of the DPPH and ABTS assays, the ethyl acetate extracts of the stem bark showed the strongest scavenging activity with TEAC values of 1195.57 ± 3.33 and 640.57 ± 10.98 , respectively. Nevertheless, the hexane extract from the leaves was the weakest radical scavenger when evaluated by DPPH and ABTS.

In CUPRAC, the strongest antioxidant activity was possessed by the ethyl acetate extract of the leaves, followed by the leaf chloroform extract and ethyl acetate extract of the stem bark, with TEAC values of 902.78 ± 26.31 , 843.10 ± 69.05 and 822.48 ± 59.07 , respectively. In contrast, the hexane extract of the leaves showed the weakest CUPRAC activity with a TEAC value 553.39 ± 46.95 . In FRAP, the antioxidant activities of the extracts were increased with the increasing polarity of the extraction solvent used. The antioxidant activity of the

extracts followed the trend of hexane < chloroform < ethyl acetate. The polar (ethyl acetate) extract exhibited higher antioxidant capacity than non-polar (hexane and chloroform). These findings were supported by studies conducted in Thailand and India in which polar extracts also exhibited better antioxidant activities. From our data analysis, extracts of *Aegle marmelos* were found to possess more powerful reducing ion ability compared to free radical scavenging.

ANTIOXIDANT CAPACITIES OF *MURRAYA KOENIGII* AS MEASURED BY DPPH, ABTS, FRAP AND CUPRAC ASSAYS

Figure 3 illustrates the antioxidant activities of various *Murraya koenigii* extracts measured by different antioxidant assays. All the root extracts were significantly active in FRAP but gave weak antioxidant activity in DPPH assay except the ethyl acetate extract from the root. Meanwhile, the stem bark extracts showed strong antioxidant activities based on CUPRAC but was a weak antioxidant when measured by ABTS. This might be due to the nature of the phytochemicals present in the extracts which are more favorable in the electron transfer mechanism to stabilize the cupric ion rather than quench the radical chain reaction in DPPH assay (Tachakittirungrod et al. 2007).

The stem bark chloroform extract had the strongest scavenging effects towards DPPH• followed by the stem bark ethyl acetate extract which gave the TEAC values of 1530.16 ± 309.90 and 1359.33 ± 421.53 , respectively. *Murraya koenigii* exhibited moderate antioxidant activity in the ABTS assay. The hexane root extract was found to possess the highest activity against ABTS with a TEAC value of 904.45 ± 25.61 . Meanwhile, the chloroform extract from the roots (634.53 ± 29.27) showed the weakest antioxidant activity. The high antioxidant activity shown by the extract might be due to the chemical constituents which are chemically reactive towards the ABTS radical cation. Similar studies by Jagan Mohan Rao et al. (2007)

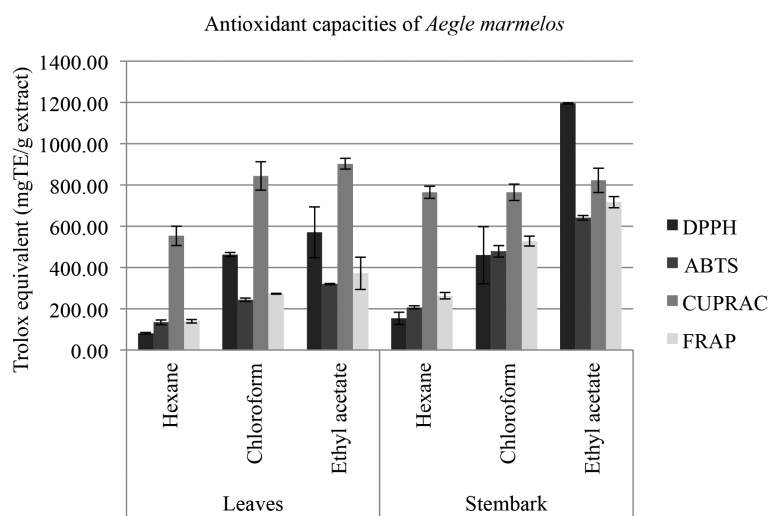


FIGURE 2. Comparison of antioxidant capacities of *Aegle marmelos*

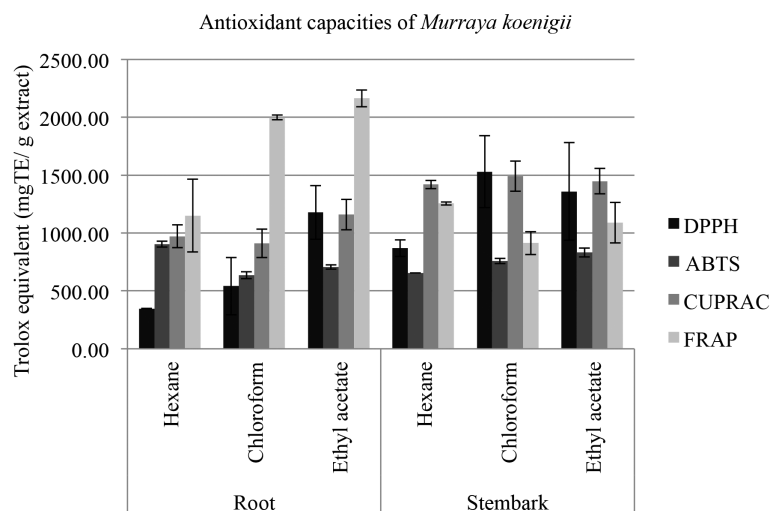


FIGURE 3. Comparison of antioxidant capacities of *Murraya koenigii*

and Ningappa et al. (2008) conducted in India also reported *Murraya koenigii* as a good antioxidant plant.

The ethyl acetate extract of the root had the highest ferric ion reducing power with TEAC values of 2163 ± 72.71 . However, the chloroform extract from the stem bark possessed the weakest ferric ion reducing power with a TEAC value of 913.14 ± 98.75 . Total phenolic content (TPC) and antioxidant activities of 5 types of Malay salads were investigated using DPPH and FRAP assays. In this study, leaves of *Murraya koenigii* were found to have the highest TPC, but it only showed moderate antioxidant activities in both DPPH and FRAP assays when compared to *Selom* and *Ulam Raja* (Reihani & Azhar 2012).

Based on antioxidant measurement by the CUPRAC assay, the extract of the stem bark showed stronger activity than the root extracts. The most ethyl acetate extract from stem bark exhibited highest CUPRAC activity with a TEAC value of 1490.89 ± 129.72 . The high values obtained from the CUPRAC assay indicated that the compounds present in the extracts were good electron donors and were able to terminate the oxidation chain reaction (Tachakittirungrod et al. 2007). However, the chloroform extract from the roots had the weakest antioxidant activity when measured by CUPRAC with a TEAC value of 911.46 ± 124.21 . The extracts from roots and stem bark showed different antioxidant activities in each measured assay, the variation of activity was probably due to the different composition of chemical constituents present in the extract. Different composition of constituents contributed to different activity of an extract. The studies on the bioactivity of propolis by Pereira et al. stated that the biological activities of the propolis are strongly related to their chemical constituents (Gil-González et al. 2013).

β-CAROTENE/LINOLEIC ACID BLEACHING ASSAY OF *AEGLE MARMELOS* AND *MURRAYA KOENIGII*

Figure 4 shows the strength of lipid peroxidation inhibition of all the extracts of both plants evaluated by linoleic

acid coupled with β-carotene bleaching assay. The results showed that all the extracts from *Murraya koenigii* species gave significant lipid peroxidation inhibitory ability as good as the standards used in the experiment. In brief, lipid peroxidation inhibitory ability of *Aegle marmelos* extracts was weaker than *Murraya koenigii* extracts. The strongest activity was shown by the hexane extract of *Murraya koenigii* roots followed by the chloroform extract of *Murraya koenigii* stem bark with values of $97.46 \pm 2.27\%$ and $93.34 \pm 4.23\%$, respectively. The hexane extract of *Aegle marmelos* stem bark was found to be pro-oxidant with an inhibition value of $-6.87 \pm 3.72\%$. There are studies which showed that some antioxidants such as α-tocopherol and ascorbic acid, act as pro-oxidants under certain doses and experimental circumstances (Zhang & Omaye 2001). Several research studies have figured out that many phenolic compounds commonly present in foods and medicinal plants such as curcumin and EGCG can exert both chemopreventive and anticancer effects due to their unique ability to have dual effect on the cellular redox status. They seem to promote antioxidant actions in order to prevent carcinogenesis and act as pro-oxidants to kill cancer cells (León-González et al. 2015). All the extracts from *Aegle marmelos* species were reported to be weaker than the standards used in this assay such as BHT, BHA and Vitamin E.

ANTIOXIDANT ACTIVITIES OF ISOLATED CONSTITUENTS AS MEASURED BY DPPH, ABTS, CUPRAC AND FRAP ASSAYS

Table 1 shows the antioxidant activities of the isolated compounds at the concentration of 1 mg/mL. Unfortunately, only four compounds namely marmin (1), 7-hydroxycoumarin (2), girinimbine (6) and mahanimbine (7) were screened due to insufficient amounts of samples. Mahanimbine (7) is the most active antioxidant based on ABTS, CUPRAC and FRAP assays (Table 1). The results indicated that mahanimbine (7) acts as a good electron donor by reducing the oxidized intermediates into

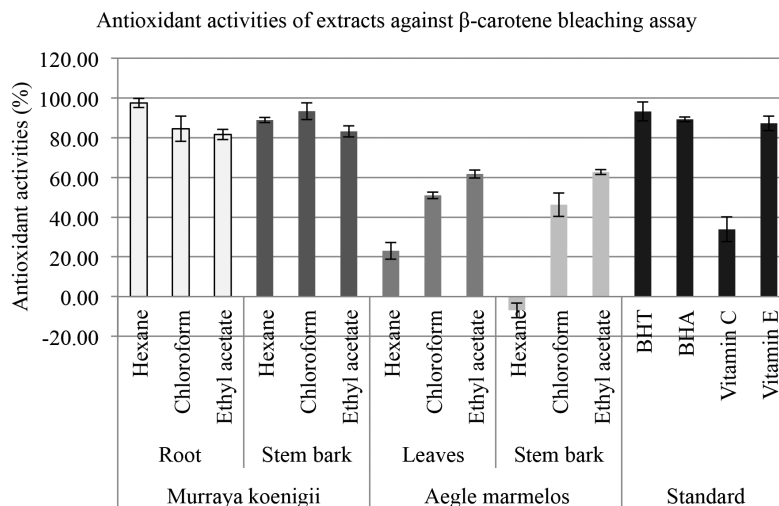


FIGURE 4. Antioxidant activities of two *Rutaceae* plants assessed by linoleic acid / β -carotene bleaching assay at concentration of 1 mg/mL

TABLE I. Antioxidant capacities of isolated compounds in TEAC

Compounds	Trolox equivalent antioxidant capacity (TEAC) ^a			
	DPPH	ABTS	CUPRAC	FRAP
marmin (1)	218.90 ± 15.00	62.78 ± 10.98	266.93 ± 6.51	117.02 ± 10.85
7-hydroxycoumarin (2)	187.09 ± 3.33	92.10 ± 20.73	217.01 ± 33.41	13.04 ± 2.71
girinimbine (6)	162.35 ± 5.00	880.30 ± 71.95 ^b	377.60 ± 155.57	690.61 ± 96.58 ^b
mahanimbine (7)	85.77 ± 3.33	927.73 ± 0.00 ^b	1649.31 ± 460.37 ^c	1259.21 ± 45.58 ^b

All values are expressed as mean ± standard deviation of triplicates

^a mg TE/ g compound

^b indicates significantly different ($p < 0.0001$)

^c indicates significantly different ($p < 0.001$)

their stable form to purge the oxidation chain reaction (Tachakittirungrod et al. 2007). This might be due to the electron rich properties of the isoprene unit present in mahanimbine (7) which leads to a high tendency in the single electron donating mechanism which enhances the antioxidant capacities of mahanimbine (7) in metal ion reducing assays. However, mahanimbine (7) showed the weakest activity in DPPH. In the previous studies, the antioxidant compounds, mahanimbine (7) and koenigine were evaluated through DPPH radical scavenging activities where koenigine showed a significant activities with the scavenging percentage of 91.6%, but mahanimbine (7) showed a weak radical scavenging activity with the value of 18.8% (Jagan Mohan Rao et al. 2007). Shimada et al. (1992) suggested the radical-scavenging activity of the compounds were due to the hydrogen-donating ability. This occurrence may be caused by the weak hydrogen-donating ability of mahanimbine (7). Mahanimbine (7) could also be one of the compounds responsible for the strong antioxidant activity observed in the stem bark of chloroform extract.

The antioxidant strengths of the isolated compounds based on DPPH assays is in the order of marmin (1) > 7-hydroxycoumarin (2) > girinimbine (6) > mahanimbine

(7). The presence of -OH substituent group in marmin (1) and 7-hydroxycoumarin (2) allows these compounds to be more active antioxidants than the carbazole alkaloids as a radical scavengers. López et al. (2003) stated that the presence of multiple hydroxyl substituents groups in the polyphenols make them idea for free radical scavenging reaction and as metal chelating agents. In addition, the chemical substituent functional group which plays an important role in the reaction with the free radical was identify to be the hydroxyl (-OH) group (Bendary et al. 2013). Besides, the arrangement of the hydroxyl group around the phenolic molecules is also important in the antioxidant activities (Moure 2001). Meanwhile, the absence of hydroxyl (-OH) functional group in both mahanimbine (7) and girinimbine (6) reduces their anti-radical ability. In ABTS, mahanimbine (7) had the strongest antioxidant activity. In contrast, marmin (1) was the weakest antioxidant agent among the four tested compounds with a TEAC value of 62.78 ± 10.98. In ABTS assay, the mechanism of the quenching of ABTS radical cation involves single electron transfer. Since mahanimbine (7) has a high electron density in the isoprene unit side chain, it acts as a good electron donor to purge the radical species.

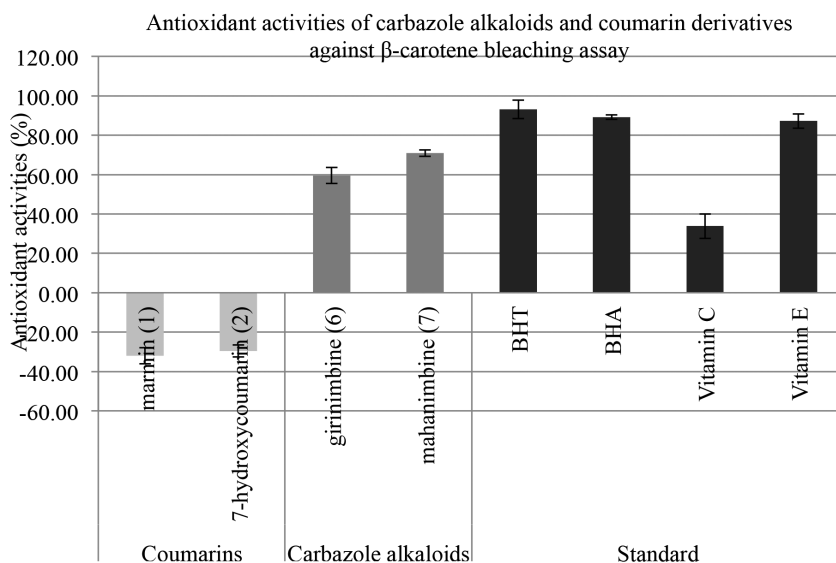


FIGURE 5. Antioxidant activities of isolated compounds assessed by linoleic acid / β -carotene bleaching assay

Figure 5 illustrates the antioxidant activity of isolated compounds assessed by linoleic acid/ β -carotene bleaching assay. Again, mahanimbine (7) exhibited the strongest inhibition followed by girinimbine (6) towards the lipid peroxidation of linoleic acid in the β -carotene bleaching assay. The inhibitory percentages were recorded as $70.95 \pm 1.66\%$ and $59.54 \pm 4.02\%$, respectively. Jayaprakasha et al. (2001) stated that the extent of β -carotene bleaching was hindered by the presence of an antioxidant agent, which neutralizes the linoleate-free radical generated in the linoleic acid/ β -carotene system. Girinimbine (6) and mahanimbine (7) has shown to have significant abilities to neutralize the hydroperoxide radical and inhibit the oxidation of β -carotene. The antioxidant activity of the isolated compounds based on β -carotene assay can be ranked as follows: mahanimbine (7) > girinimbine (6) > marmin (1) > 7-hydroxycoumarin (2).

CONCLUSION

Aegle marmelos and *Murraya koenigii* are two Rutaceae plants which showed good antioxidant activities. The antioxidant activity of extracts from *Murraya koenigii* is stronger than that of extracts from *Aegle marmelos* in both free radical scavenging activities and metal ion reducing ability. The active antioxidant activity of the isolated bioactive secondary metabolites from these plants particularly girinimbine (6) and mahanimbine (7) may suggest that these two compounds could be the chemical markers for the antioxidative property of the *Aegle marmelos* and *Murraya koenigii*. Mahanimbine (7) which is the most potent bioactive compound based on CUPRAC, FRAP and ABTS can be transformed into an antioxidant agent but have yet to be further investigated *in-vivo* and by cellular antioxidant measurement in future.

Although various researches had shown both antioxidant and pro-oxidant properties of a polyphenol-rich food were beneficial in cancer treatment yet more studies regarding the interactions between anti- or pro-oxidants and ROS in human are needed.

ACKNOWLEDGEMENTS

The authors thank Dr. Sugeng Riyanto from University of Gadjah Mada, Yogyakarta, Indonesia for his valuable help in supplying the plant samples (*Aegle marmelos*). Deepest gratitude is extended to Ministry of Education (MOE), Malaysia for Mybrain15 scholarship to one of the authors and IPS-Putra grant (Vot no. 9461100) for financial support in the study.

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Received: 29 August 2017

Accepted: 21 March 2018