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RESEARCH ARTICLE

Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae)

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ABSTRACT

Phytochemicals and biological activities of the leaves of *Macaranga hosei* and *Macaranga constricta* have been studied. Fractionation and purification of the extracts of *M. hosei* afforded two triterpenoids, lupenone **(1)** and β-sitostenone **(2)** and two flavonoids, 5-hydroxy-7,4'-dimethoxyflavone **(3)** and 5-hydroxy-6,7,4'-trimethoxyflavone **(4)**. Three triterpenoids characterized as taraxerone **(5)**, taraxerol **(6)** and β-amyrin **(7)** were isolated from *M. constricta*. The structures of these compounds were established by analysis of their spectroscopic data, as compared to that of reported compounds. Biological activities which include antibacterial, α-glucosidase inhibition and antioxidant were also carried out. The antibacterial activities have demonstrated that all extracts and isolated compounds exhibited weak inhibition against the tested bacterial strains with MIC value exceeded 500 µg/mL. Evaluation of α-glucosidase inhibition activity using *ρ*-nitrophenyl-*ρ*-D-glucopyranosidase on extracts exhibited α-glucosidase inhibitory potential. The most potent α-glucosidase activity was exhibited by the petroleum ether extract of *M. hosei* with inhibitory concentration at 50% (IC_{50}) of 25.3 ppm compared with quercetin (4.5 ppm) and acarbose (12.6 ppm). The antioxidant activity was conducted through DPPH radical scavenging activity and total phenolic content. All the extracts displayed positive results and the methanol extract of *M. hosei* displayed the highest scavenging activity with scavenging concentration at 50% (SC_{50}) value of 25.8 ppm. The methanol extract of *M. hosei* also gave the highest total phenol content with 347.7 mg GAE/g.

Key words: *Macaranga hosei*; *Macaranga constricta*; antibacterial; α-glucosidase; antioxidant

1. Introduction

Plants of *Macaranga* genus is the only genus in the subtribe Macaranginae with over 300 species. This genus is commonly grown in secondary forest and newly cleared areas and distributed in tropical Africa, Madagascar, centered in South-East Asia, Australia and the Pacific regions [1-2]. Approximately, 40 species are reported to be found in Malaysia's secondary forest [2]. The species of the genus ranging from shade tolerant understory shrubs and small trees to light demanding pioneers [3]. Among the species reported in Malaysia, nine of them are myrmecophytes or mutualistics ant-plants [4-5]. This symbiosis relationship occurs when the plants provide housing or shelter and also food for specific ants in exchange for the protection from herbivorous insects [4]. Two *Macaranga* species have been selected for the current phytochemical investigation namely *M. hosei* King ex. Hook.f. and *M. constricta* Whitmore & Airy Shaw.

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Macaranga hosei is a large tree that can grow up to 20 to 30 m tall with a bluishgreen appearance. It is distributed in Peninsular Thailand, Peninsular Malaysia, Sumatra and Borneo and commonly recognized as an early successional tree in lowland areas. The twigs of this plant are becoming hollow due to ant colonization [6]. This myrmechophytic species also have huge, alternate yet simple leaves with three or more veins on it. *Macaranga constricta* is distributed in Peninsular Malaysia specifically in northeast of Gunung Benom, Pahang [7]. It is a dull grayish-green tree which can grow up to 27 metre tall and can be found in the middle of lowland rain-forest canopy on the hillsides [6-7]. This myrmechophytic species can be recognized by its secondary veins which are strongly encircle and the swelling parts of the twigs become narrower when dry while the complete male flower part of the plant usually have smooth and even surface [8].

Plant species of genus *Macaranga* have been used as traditional medicines to treat fungal infections, stomachaches, reduce fever, coughs and tonsillitis. This medicinal uses highlight the need to study the phytochemicals and biological activities of *M. hosei* and *M. constricta* before the extinction of these species due to deforestation for rural development. Among the species only *M. hosei* have been studied and reported. Isoprenylated flavanones were reported from *M. hosei* collected from Kalimantan, Indonesia [9]. No phytochemicals or biological activities have been reported from *M. constricta*. This research will provide database of phytochemicals and bioactivities of two *Macaranga species*. The findings obtained from this research would contribute to the fields of medicine and development of nutraceutical product.

2. Results and Discussion

The medicinal values possessed by plant of *Macaranga* genus have led to phytochemical studies by many researchers. It has been reported that hundreds of secondary metabolites have been successfully isolated. In this study, phytochemical investigation on the leaves of two *Macaranga* species has been investigated. Four compounds have been isolated from *M. hosei* which are lupenone **(1)** [10], β-sitostenone **(2)** [11], 5-hydroxy-7,4′-dimethoxyflavone **(3)** [12], and 5-hydroxy-6,7,4′-trimethoxyflavone **(4)** [13]. In addition, taraxerone **(5)** [14], taraxerol **(6)** [15], and β-amyrin **(7)** [16] have been isolated from *M. constricta*. These metabolites were identified by analysing their spectroscopic data and comparing them with the literature data. Only one phytochemical study on *M. hosei* has been reported previously. Two isoprenylated flavanones namely 4'-*O*-methyl-8-isoprenylnaringenin and lonchocarpol A have been isolated from the methanol extract of the leaves of *M. hosei*, collected from Indonesia [9]. The current phytochemical investigation provided different metabolites from the species of Indonesia. Flavonoids also have been isolated previously from various *Macaranga* species such as *M. tanarius* [17], *M. triloba* [18], *M. indica* [19], *M. vedeliana* [20], *M. denticulate* [21], and *M. kurzii* [22].

Lupenone **(1)** - White solid (2.0 mg, 5.4%); m.p. 164-166°C; IR ν_{max} cm^{-1}: 2916, 1714, 1679; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.60 (1H, d, *J* = 2.4 Hz, H-29α), 4.72 (1H, d, *J* = 2.4 Hz, H-29β), 2.80 (1H, m, H-19), 2.30 (2H, m, H-21), 1.40-2.00 (22H, m, H-1/H-2/H-5/H-6/H-7/H-9/H-11/H-12/H-13/H-15/H-16/H-18/H-19/H-22), 1.27 (12H, s, H-24/H-23/H-26/H-30), 1.17 (3H, s H-25), 0.93 (3H, s, H-27), 0.83 (3H, s H-28); GC-MS: *m/z* 424 [10].

β-Sitostenone **(2)** - Colourless solid (43.4 mg, 8.7%); m.p. 95-97°C; IR ν_{max} cm^{-1}: 2926, 1715, 1658; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (1H, s, H-6), 1.00-2.40 (29H, m, H-1/H-2/H-4/H-7/H-8/H-9/H-11/H-12/H-14/H-15/H-16/H-17/H-20/H-22/H-23/H-24/H-25/H-28), 0.90 (H-19), 0.89 (H-29), 0.87 (H-27), 0.84 (H-26), 0.82 (H-21), 0.73 (H-18); GC-MS: *m/z* 412 [11].

5-Hydroxy-7,4′-dimethoxyflavone **(3)** - Yellow solid (1.9 mg, 11.7%); m.p. 252-254°C; IR ν_{max} cm^{-1}: 3298, 1659, 1594, 1502, 1115; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.77 (1H, s, H-5), 8.20 (2H, d, *J* = 9.20 Hz, H-2'/H-6'), 7.06 (2H, d, *J* = 9.20 Hz, H-3'/H-5'), 6.64 (1H, s, H-3), 6.58 (1H, d, *J* = 2.40 Hz, H-6), 6.40 (1H, d, *J* = 2.40 Hz, H-8), 3.90 (6H, s, 2×OCH_3); EIMS: *m/z* 298 [12].

5-Hydroxy-6,7,4′-trimethoxyflavone **(4)** - Yellow solid (2.0 mg, 5.4%); m.p. 280-282°C; IR ν_{max} cm^{-1}: 3334, 1649, 1602, 1504, 1217; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.69 (1H, s, H-5), 8.10 (2H, d, *J* = 9.20 Hz, H-2'/H-6'), 7.06 (2H, d, *J* = 9.20 Hz, H-3'/H-5'), 6.48 (1H, s, H-3), 6.38 (1H, s, H-8), 3.90 (9H, s, 3×OCH_3); EIMS: *m/z* 328 [13].

Taraxerone **(5)** - White solid (0.14 g, 67.5%); m.p. 240-242°C; IR ν_{max} cm^{-1}: 2934, 1708, 1641; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.58 (1H, dd, *J* = 3.6, 8.4 Hz, H-15), 2.60 (1H, m, H-2β), 2.35 (1H, ddd, *J* = 3.6, 6.4, 16.0 Hz, H-2α), 2.10 (1H, dt, *J* = 3.6, 13.2 Hz, H-19β), 1.94 (1H, dd, *J* = 3.2, 15.2 Hz, H-16β), 1.25-1.72 (21H, m, H-1/H-5/H-6/H-7/H-9/H-11/H-12/H-16α/H-18/H-19α/H-21/H-22), 1.16 (3H, s, H-27), 1.10 (9H, s, H-23/H-25/H-24), 0.98 (3H, s, H-29), 0.93 (6H, s, H-28/H-30), 0.85 (3H, s, H-26); GC-MS: *m/z* 424 [14].

Taraxerol **(6)** - White solid (26.6 mg, 2.2%); m.p. 276-278°C; IR ν_{max} cm^{-1}: 3444, 2928, 1632, 1233; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.56 (1H, dd, *J* = 3.2, 8.4 Hz, H-15), 3.22 (1H, dd, *J* = 4.8 and 11.2 Hz, H-3), 2.05 (1H, m, H-19β), 1.94 (1H, dd, *J* = 3.2 and 14.8 Hz, H-16β), 1.60 (21H, m, H-2, H-7, H-16α, H-1/H-11/H-6/H-18/H-22/H-19α/H-12/H-21), 1.11 (3H, s, H-27), 1.00 (3H, s, H-23), 0.97 (3H, s, H-29), 0.95 (3H, s, H-24), 0.93 (3H, s, H-26), 0.90 (3H, s, H-30), 0.84 (3H, s, H-28), 0.83 (3H, s, H-25); GC-MS: *m/z* 426 [15].

β-Amyrin **(7)** - White solid (22 mg, 22%); m.p. 196-198°C; IR ν_{max} cm^{-1}: 3445, 2932, 1636, 1227; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.54 (1H, dd, *J* = 3.2, 8.0 Hz, H-12), 3.42 (1H, t, *J* = 2.8 Hz, H-3), 1.99 (3H, m, CH, H-5/H-9/H-14), 1.49 (20H, m, H-1/H-2/H-6/H-7/H-11/H-15/H-16/H-19/H-21/H-22), 1.10 (3H, s, H-27), 0.95 (9H, s, H-24/H-26/H-28), 0.92 (6H, s, H-29/H-30), 0.88 (3H, s, H-23), 0.83 (3H, s, H-25); 3); EIMS: *m/z* 426 [16].

Macaranga species have shown a lot of potential as natural medicines as they had been used in traditional medicine by native people from respective places. Therefore, a lot of studies have been conducted on various species to prove their medicinal values. In this study, the biological activities which had been reported are antioxidant, α-glucosidase inhibitory and antibacterial activities. Total phenolic content was determined using Folin-Ciocalteau's reagent adapted to a 96-well microplate where gallic acid was used as the standard. The results were expressed as mg gallic acid per gram of extract (mg GAE/g) [23]. Table 1 displays the results of total phenolic contents of all extracts. The MeOH extract of *M. hosei* showed the highest phenolic content (347.7 mg GAE/g) compared to other extracts. The results of total phenolic content obtained from these two extracts correlated with the antioxidant values resulted in free radical scavenging assay against DPPH. This proved the theory that the phenolic compounds may be present in these extracts which helps in enhancing the antioxidant values [23]. From observation, the total phenolic content of extracts increased with the increasing polarity of solvents. The summary of the SC_{50} values of extracts against DPPH are presented in Table 1. Among all the extracts from two *Macaranga* species, the MeOH extract from *M. hosei* displayed the most significant free radical scavenging activity with SC_{50} value of 25.8 ppm. This is followed by the MeOH extract of *M. constricta* and the $CHCl_3$ extract of *M. hosei* with SC_{50} values of 26.7 and 31.4 ppm respectively. The high antioxidant activity displayed by these two extracts might be resulted from the presence of phenolic compounds with high number of hydroxyl groups which can donate hydrogens to stabilize the free radicals [23].

α-Glucosidase inhibitory assay was conducted on all extracts of the *Macaranga* species. In this assay, the inhibitors present in the body suppressed α-glucosidase enzyme and inhibited the release of glucose from the carbohydrate which later reduced the postprandial blood glucose level [24]. The assay was determined spectrophotometrically at 405 nm in a 96-well microtiter plate. The results of the α-glucosidase inhibition activity of the tested samples are summarized in Table 1**.** The results showed that all crude extracts exhibited positive result as α-glucosidase inhibitors with petroleum ether extract of *M. hosei* exhibited the most potent activity with IC_{50} 25.3 ppm. It was followed closely by the MeOH and $CHCl_3$ extracts of the same species each with IC_{50} 36.0 and 44.2 ppm. The PE extract of *M. constricta* exhibited the least inhibition activity with IC_{50} 237.6 ppm. The high inhibition activity of *M. hosei* extracts may be contributed by the presence of high content of phenolic compounds which were

Table 1. Percentage yield, antioxidant and a-glucosidase inhibitory activity of *Macaranga* species

Species	Extract	Yield (g, %)	TPC (mg GAE/g)	DPPH SC_{50} (ppm)	α-Glucosidase IC_{50} (ppm)
M. hosei	PE	12.0 g, 2.4%	164.8 ± 16.8	73.1 ± 30.4	25.3
	$CHCl_3$	0.3 g, 0.1%	198.9 ± 29.9	31.4 ± 4.2	44.2
	MeOH	17.5 g, 3.5%	347.7 ± 9.2	25.8 ± 1.7	36.0
M. constricta	PE	7.3 g, 1.5%	148.4 ± 3.7	>1000	237.6
	$CHCl_3$	4.9 g, 1.0%	152.4 ± 22.0	178.6 ± 12.8	56.1
	MeOH	30.0 g, 6.0%	316.7 ± 47.7	26.7 ± 1.5	91.9

PE – petroleum ether; GAE - gallic aicd equivalent; Standards - Ascorbic acid (SC_{50} 8.9 ± 0.2 ppm); Butylated hydroxyl toluene (SC_{50} 15.3 ± 0.6 ppm); Quercetin (IC_{50} 4.5 ppm); Acarbose (IC_{50} 12.6 ppm)

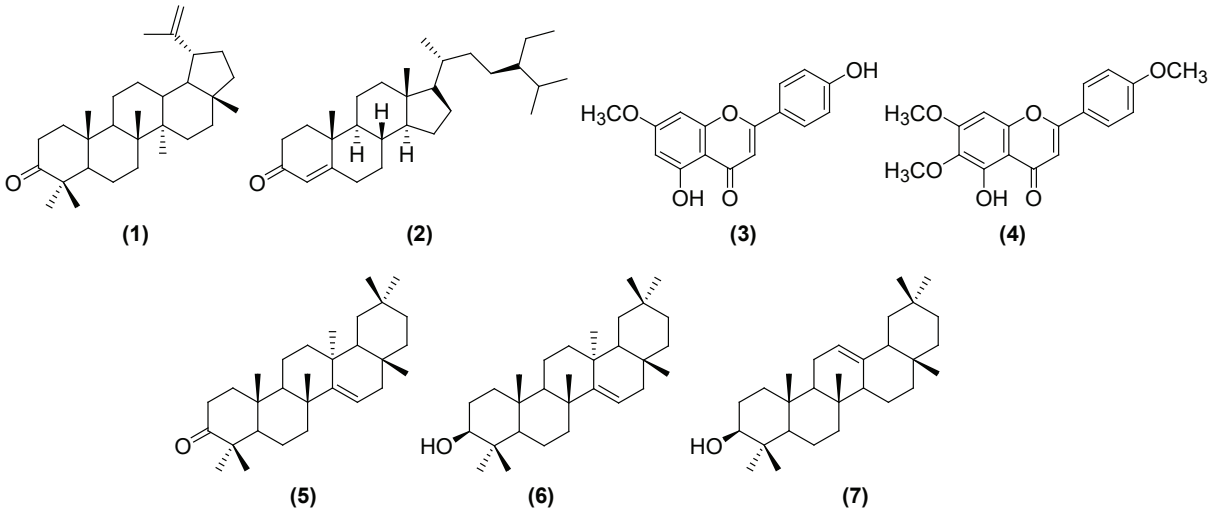

Figure 1. Chemical structures of compounds isolated from *Macaranga* species

failed to be isolated in this study. Phenolic compounds can be oxidized to phenolate ions or quinones, thus, they are able to block or enhance responsible's enzymes for the digestion of carbohydrate [25].

The antibacterial assay was conducted on the extracts using micro dilution method. None of the extracts were effective against *B. subtilis*, *S. aureus* and *E. coli*. Meanwhile, all the isolated compounds displayed no inhibition activity against all three bacterial strains. This suggested that all compounds do not have antibacterial properties which may correlate with weak activity observed on the extracts. Previous studies revealed the isolation of triterpenoids derivatives with significant antibacterial properties identified as arjulonic acid and asiatic acid from *Syzygium guineense* [26]. The presence of hydroxyl group at C-23 and carboxylic acid substituent in the structure was the determining factors which aids in the antibacterial properties of these compounds against variety of pathogenic bacteria [26]. Thus, the absence of these acid substituents in the structure of triterpenoids **(5)**, **(6)**, and **(7)** may explains the absence of inhibition towards the tested bacterial strains. A study conducted on *Chromolaena* species by Taleb-Contini *et al*., [27] had revealed the isolation of some flavonoids derivatives with significant antibacterial properties. It is revealed that flavonoids with hydroxyl groups at C-7, C-3' and C-4' positions gave positive antibacterial tests. This result correlated with the weak antibacterial activity possessed by compound **(3)** where it has only methoxyl groups at position C-7 and C-4' which may decrease the antibacterial property.

3. Conclusion

In conclusion, very little phytochemical work has been done on the genus *Macaranga*. An overview of the literature search indicated that only twenty six *Macaranga* species have been investigated phytochemically as compared to about 300 plant species known from this genus. Therefore, more phytochemical studies are needed to validate the claimed of their traditional uses.

4. Materials and Methods

4.1 General experimental procedures

Two types of extraction methods were applied which were cold extraction and soxhlet extraction techniques. All the chemicals were analytical grade. Petroleum ether (PE), *n*-hexane, diethyl ether (Et_2O), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), ethyl acetate (EtOAc) and methanol (MeOH) were the solvents used in the extraction and chromatographic process. Preliminary phytochemical screening was conducted on all extracts. Evaporation of the solvents from the crude extracts was carried out using Eyela Rotary Evaporator N-1000 model. Thin Layer Chromatography (TLC) analysis was done on precoated silica gel aluminium sheets with Merck Silica Gel $60F_{254}$ with the thickness of 0.2 mm as the stationary phase. The spots on the TLC plates were visualized by UV at 254 nm and 365 nm before sprayed with vanillin sulphuric acid reagent. Further fractionation and purification of the crude extracts were conducted using vacuum liquid chromatography (VLC)

and column chromatography (CC). Merck silica gel (SiO_2) of 230-400 Mesh was used as the stationary phase for VLC while SiO_2 of 70-230 Mesh was the packing material for CC. Melting points were measured using a hot stage Gallen apparatus and were uncorrected. Infrared Spectroscopy (IR) was recorded on a Perkin Elmer FTIR Frontier spectrophotometer with Universal ATR sampling accessory. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance 400 and 100 MHz spectrometer using deuterated solvent $CDCl_3$ or stated otherwise. Mass spectral data were acquired by GC-MS and Bruker Mass Spectrometry Services from National University Singapore. Gas chromatography (GC) was performed using a Hewlett Packard HP6890 series II A fitted with Ultra-I column. GC-MS analyses were carried out using a Hewlett Packard Model 5890A gas chromatograph and a Hewlett Packard Model 5989A mass spectrometer.

4.2 Plant materials

The fresh leaves of *M. hosei* (HTBP 290) and *M. constricta* (HTBP 1121) were collected from Terengganu in 2007 and identified by Mr. Ahmad Zainuddin Ibrahim. The voucher specimens were deposited at the herbarium of Universiti Kebangsaan Malaysia.

4.3 Extraction and isolation

The dried leaves of *M. hosei* (500 g) were ground into powder and extracted with soxhlet apparatus using PE (5 L), $CHCl_3$ (5 L) and MeOH (5 L) consecutively for 16 h. The PE extracts (11.0 g) was subjected to VLC (*n*-hexane:Et_2O) on SiO_2 230-400 mesh to give five major fractions, MHP 1-5. The MHP 2 fraction (55.0 mg) was purified using CC (*n*-hexane:Et_2O) in stepwise gradient. Fractions with similar TLC profiles were combined to obtain five major fractions MHP 2A-2E. Fraction MHP 2E was further purified using SiO_2 CC (*n*-hexane:Et_2O) and fractions 1-19 were combined to afford lupenone **(1)**. Fractions MHP 4 (4.0 g) was purified by CC over SiO_2 to afford a total of 400 fractions collected and combined to give seven subfractions, MHP 4A-G. Subfraction MHP 4A (0.5 g) was further purified by CC (*n*-hexane:Et_2O) to yield β-sitostenone **(2)**. MHP 4B (13.7 mg) was purified by CC (*n*-hexane:Et_2O) in stepwise gradient to yield 240 fractions. Fractions 101-141 were combined and concentrated to give 5-hydroxy-7,4'-dimethoxyflavone **(3)**. The $CHCl_3$ extract (0.3 g) was subjected to CC on SiO_2 (*n*-hexane:Et_2O) in increasing polarity to give 180 fractions. Further purifications on the fractions failed to give a pure compound. The MeOH extract (17.45 g) was subjected to VLC (*n*-hexane:EtOAc:acetone) in increasing polarity to give five fractions, MHM 1-5. Fraction MHM 2 (0.1 g) was then subjected to CC (*n*-hexane:$CHCl_3$) to give a total of 100 fractions which were combined to give five major fractions labeled as MHM 2A-E. Subfraction MHM 2A (0.04 g) undergone further purification over CC on SiO_2 with *n*-hexane-EtOAc as the eluents mixture. Fractions 1-95 from MHM 2-A were combined and concentrated to give 5-hydroxy-6,7,4'-trimethoxyflavone **(4)**.

The dried leaves of *M. constricta* (500 g) were ground into powder and extracted with soxhlet apparatus using PE (5 L), $CHCl_3$ (5 L) and MeOH (5 L) consecutively for 16 h. The PE extracts (MCP) (7.0 g) was subjected to VLC on SiO_2 (230-400 mesh) with *n*-hexane and Et_2O in stepwise gradient were used as the eluents to give twelve major fractions labeled as MCP1-12. Upon removal of solvent, fraction MCP 3 (0.14 g) was recrystallized with *n*-hexane repetitively to yield taraxerone **(5)**. Fraction MCP 7 (0.1 g) was washed with *n*-hexane repetitively to give β-amyrin **(7)**. Fraction MCP 12 (1.2 g) was subjected to CC on SiO_2 (70-230 mesh), using gradient elution of *n*-hexane:Et_2O to give a total of 245 fractions. Fractions were combined based on TLC profile to give eight new fractions labeled as MCP 12-A to MCP 12-H where fraction MCP 12-C (26.6 mg) yielded taraxerol **(6)**. The $CHCl_3$ extracts (MCC) (4.5 g) was fractionated on VLC using using *n*-hexane and Et_2O in increasing polarity as the eluent mixture. TLC screening was conducted on the fractions obtained and similar profiles of TLC was combined into five major fractions and labeled as MCC 1-5. Fraction MCC 2 was purified repetitively on CC using *n*-hexane and Et_2O eluents in stepwise gradient. A single spot was obtained from this fraction and ^1H NMR analysis on the sample showed that it was similar compound to compound **(6)**. The MeOH extracts (MCM) (25.0 g) was fractionated using *n*-hexane:Et_2O:EtOAc on VLC to give four major fractions labeled as MCM 1-4. However, purification failed to isolate a pure compound from these fractions.

4.4 Biological activities

Antioxidant: 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, gallic acid, and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich (Germany). Analytical grade methanol, ethanol and dimethylsulfoxide (DMSO) were purchased from Merck (Germany). ***α-Glucosidase:*** Dimethyl sulfoxide (DMSO), p-nitrophenyl-α-D-glucopyranose (PNPG), α-glucosidase (Maltase) enzyme, acarbose and quercetin hydrate were purchased from

Sigma-Aldrich (Germany). Potassium phosphate monobasic (KH_2PO_4) and sodium carbonate (Na_2CO_3) were purchased from Merck (Germany). ***Antibacterial:*** Nutrient agar (NA), nutrient broth (NB), and streptomycin sulphate were purchased from Oxoid (Italy). All tested microorganisms were purchased from Mutiara Scientific (Malaysia).

4.5 Antioxidant activity

4.5.1 Total phenolic content (TPC)

Total phenolic content was carried out according to the method described by Kassim et al. [28] with minor modifications. The stock solution of 1000 ppm of the tested sample and standard Gallic acid was prepared. Sodium carbonate solution was prepared by dissolving 3 g of sodium carbonate with 50 mL of distilled water. 40 µL of stock solution was added into the first two rows (A and B) before double manifold dilution was conducted by mixing the solution with 40 µL of MeOH from row B to H. Then, 20 µL of Follin-Ciocaltaeu's reagent was mixed into the solution and it was incubated for 5 mins in dark condition. After incubation, 80 µL of Na_2CO_3 solution and 60 µL of distilled water were added. The tested solution was then incubated for 90 min in dark condition before it was measured at 760 nm. A calibration graph of standard gallic acid was constructed. Total phenolic content was expressed as mg Gallic acid (GAE) per gram of extract.

4.5.2 DPPH Radical scavenging assay

The DPPH free radical scavenging assay was conducted based on method reported by Kassim et al. [28] with minor modifications. 2000 µM of DPPH reagent was prepared by dissolving 0.8 mg of DPPH in 1 mL of MeOH. The stock solution of the tested samples was prepared by dissolving it with MeOH to obtain concentration of 1000 ppm. 170 µL of stock solution was then added to the first two rows (A and B) of the 96-well microplate. Double manifold dilution was performed on row B to row H by adding 170 µL of MeOH into them. This step was conducted so that, we can have a series of concentration ranging from 1000 to 7.81 ppm. After that, 30 µL of DPPH reagent was added into all rows before incubated for 30 mins in dark condition. The absorbance of the tested solution was measured at 517 nm using Epoch microplate reader. The percentage of scavenging of DPPH was calculated using the following formula;

$$I\% = [\ A_{blank} - A_{sample} / A_{blank}\ ] \times 100$$

4.6 α-Glucosidase Inhibitory Activity

The assay was performed following the methods of Mediani et al. [29] with slight modifications. In brief, 10 µL of sample with concentration of 100, 80, 60, 40, 20, 10 and 1 µM in 5% DMSO was added to 130 µL of potassium diphosphate buffer (30 mM, pH 6.5) and 10 µL α-glucosidase enzyme (Maltase - 2 U/mL) in the 96-well plate. The mixture was incubated for 20 min. at 37°C before addition of 50 µL of *p*-nitrophenyl-*α*-*D*-glucopyranoside (*p*-NPG) (1 mM). The mixture was incubated for another 20 min. at 37°C in the dark. The reaction was quenched by adding 50 µL of sodium carbonate solution (0.2 M) and the absorbance was recorded at 405 nm by measuring the quantity of *p*-nitrophenol released from *p*-NPG. Acarbose was employed as the positive control for this assay. The percentage of inhibition was calculated as a percentage relative to a control.

$$[\ I\% = A_{Control} - (\ A_{Sample} - A_{Blank\ sample}\ ) / A_{Control} \times 100\ ]$$

where A_{Sample} is the absorbance of the reaction mixture of the test sample, $A_{Blank\ sample}$ is the absorbance of the reaction mixture containing all reagents except enzyme and $A_{Control}$ is the absorbance of the reaction mixture containing all reagents except the test sample.

4.7 Antibacterial activity

The test microorganisms, *Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (ATCC29737) and *Escherichia coli* (ATCC10536) were used. The strains were grown on Nutrient broth (NB) for the bacteria. The minimal inhibitory concentration (MIC) was determined by broth micro dilution method using 96-well microplates [30]. The inocula of the microbial strains were prepared from 24 h broth cultures and McFarland standard turbidity of suspensions was adjusted to 0.5. Sample (1 mg) was dissolved in DMSO (1 mL) to get 1000 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. The mixture of samples and sterile broth (100 µL) were transferred to each well in order to obtain a twofold serial dilution of the stock samples (concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.63 and 7.8 µg/mL). The inoculated bacteria (100 µL) were added to each well. The final volume in each well was 200 µL. Strepyomysin sulfate was used as positive controls for bacterial. Plates were incubated at 37 °C for 24 h. Microbial growth was indicated by the turbidity and the presence of pellet at the bottom of the well.

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Authorship contributions

Concept – W.M.N.H.W.S., N.Z.A.R., F.A.; Design – W.M.N.H.W.S., N.Z.A.R., F.A.; Supervision – F.A.; Resource – F.A.; Materials – F.A.; Data Collection and/or Processing – W.M.N.H.W.S., N.Z.A.R.; Analysis and/or Interpretation – W.M.N.H.W.S., N.Z.A.R., F.A.; Literature Search – W.M.N.H.W.S., N.Z.A.R.; Writing – W.M.N.H.W.S., N.Z.A.R..; Critical Reviews – W.M.N.H.W.S., N.Z.A.R., F.A.

Conflict of interest

The authors declared no conflict of interest.

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