Malays. Appl. Biol. (2022) 51(4): 87-94
https://doi.org/10.55230/mabjournal.v51i4.15

ISOLATION, IDENTIFICATION AND BIOASSAY OF FLAVONOIDS FROM *Bouea macrophylla* GRIFF.

ISNA ATHIRAH OTHMAN[1,2], NORIZAN AHMAT[1,3*], MOHD ILHAM ADENAN[4], ZURIATI ZAHARI[1,2] and AISYAH SALIHAH KAMAROZAMAN[1,3]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[2]*Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia*
[3]*Centre of Foundation Studies, Universiti Teknologi MARA, Dengkil Campus, 43800 Dengkil, Selangor, Malaysia*
[4]*Universiti Teknologi MARA Pahang, Lintasan Semarak, Bandar Jengka, 26400 Bandar Tun Razak, Pahang*
[*]*E-mail: noriz118@uitm.edu.my*

Accepted 7 October 2022, Published online 31 October 2022

ABSTRACT

Bouea macrophylla Griff., a species belonging to the Anacardiaceae family is a flowering plant native to Southeast Asia and also known as kundang, kundang daun besar, and setar in Malaysia. The fruit can be eaten raw or as a pickle, while the young leaves can be consumed as salads. It has been claimed to be able to accelerate wound healing, prevent cancer, reduce the risk of stroke, and enhance blood circulation. The previous study on the plant from the same genus, known as *B. oppositofolia* has shown the presence of various flavonoids. The present study was designed to isolate and elucidate flavonoids from this plant. The twig extract of kundang was purified by using several chromatographic techniques including Vacuum Liquid Chromatography (VLC), Column Chromatography (CC), and preparative-Thin Layer Chromatography (*p*TLC). The structures of isolated compounds were characterized by using spectroscopic methods including Nuclear Magnetic Resonance (NMR), infrared (IR), and ultraviolet (UV) spectral data, as well as comparison with the data reported in the literature. Five flavonoids were isolated and purified from the twigs of *B. macrophylla* which includes one flavanonol known as garbanzol; one flavonol which is resokaempferol; one flavandiol characterized as catechin; and two flavandiol known as mollisacacidin and guibourtacacidin. The results of the glucose uptake experiment indicated that the extract and compounds tested affected the glucose uptake rate of the insulin-resistant C2C12 cell line as compared to the standard. This is the first report describing the elucidation of the stated compounds from *B. macrophylla* as well as its glucose uptake study.

Key words: *Bouea,* chromatography, flavonoids, glucose uptake activity, NMR

INTRODUCTION

In the present study, the twigs of *B. macrophylla* have been investigated in the search for interesting compounds from tropical plants of Malaysia. This species belongs to the Anacardiaceae family and it is a flowering plant native to Southeast Asia. This plant is distributed mainly in the Malay Peninsula (Perak, Pahang, Malacca), East Coast Sumatra (Langkat), and West Java (Ng, 1989) and is also known as *kundang, kundang daun besar,* and *setar* in Malaysia. The fruit can be eaten raw or as a pickle, while the young leaves can be consumed as salads. It has been claimed to be able to accelerate wound healing, prevent cancer, reduce the risk of stroke, and be good for blood circulation. Previous studies on the other plant of the same tribe (Anacardiaceae) led to the isolation of flavonoids, terpenoids, tannic acid, and other phenolic derivatives. Several reports have validated the bioactivities of this plant such as anti-diabetic, wound healing, anti-inflammatory, antiulcer and antioxidant. Noteworthy, the genus *Bouea* only consists of two species which comprises *B. macrophylla* and *B. oppositofolia*. Nik Azmin (2017) has reported that the other plant from this genus consists of mainly flavonoids which are known to be one of the metabolite classes that possessed significant antidiabetic properties (Al-Ishaq *et al*., 2019). In pursuit of this report, this study is limited to the flavonoid class of compounds. On top of that, there is very limited literature found on the isolation work as well as the bioactivities. Hence, it is essential to isolate the chemical constituents from *B. macrophylla* to access the pharmacology of these compounds on the selected bioassays. The present study was designed to isolate and elucidate flavonoids and glucose uptake assay was also conducted on selected extract and isolates. Five flavonoids were purified and identified from the twigs of *B. macrophylla*

* To whom correspondence should be addressed

which include garbanzol (**1**), resokaempferol (**2**), catechin (**3**), mollisacacidin (**4**), and guibourtacacidin (**5**). The structures of the isolated compounds were characterized by using a spectroscopic method including NMR and comparison with previously reported data. Glucose uptake was also conducted on selected extract and compounds, which indicated that the extract and compounds showed activity on the glucose uptake rate of insulin-resistant C2C12 cell line as compared to the standard with no toxicity effect when tested on MTT assay.

MATERIALS AND METHODS

Chemicals and raw materials

All chemicals used were of industrial and analytical grade purchased from Sigma Chemical Co. (St Louis, Missouri). The twigs of *B. macrophylla* were collected at Pasir Mas, Kelantan, Malaysia, and identified by Dr. Shamsul, a botanist at Universiti Kebangsaan Malaysia (UKM), and the voucher specimens were deposited in the Universiti Kebangsaan Malaysia's Herbarium, Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor with voucher number UKMB40432.

Extraction and isolation

A total of 4 kg powdered *B. Macrophylla* twigs were macerated in 10 L hexane for 24 h, decanted to collect the solvents, and subjected to a rotary evaporator at reduced atmospheric pressure to obtain a dark brown sticky residue. These steps were repeated three times to yield 32 g hexane crude extract. The extraction steps were repeated by using ethyl acetate (EA) and methanol, successively, to obtain 25 g EA crude extract and 142 g methanol crude extract. All extracts were profiled by using TLC. EA crude extract was chosen for further purification by using conventional methods including VLC, CC, and *p*TLC. The extract was subjected to VLC, fractionated by using silica gel, and eluted with mixtures of hexane and EA in increasing order of polarity (from 100:0 and 0:100) followed by EA and methanol gradient (90:10 & 80:20) ratios to afford six fractions (1-6). Another nine subfractions (51-59) were obtained from the fractionation of fraction 5 (2.22 g) by CC with the same solvent ratios as the VLC. Further purification was done on fraction 56 (55.7 g) by using *p*TLC with a 9:1 ratio of $CHCl_3$:methanol to yield the mixture of compounds **1** and **2** (6.3 mg). Furthermore, repeated *p*TLC with an 8:2 ratio of $CHCl_3$:methanol on fraction 57 (35.3 mg) yielded three compounds including the mixture of compounds **3** and **4** (3.2 mg), as well as compound **5** (1.3 mg).

Purification and structure elucidation

The structural elucidation of the isolated compounds was done by using several spectroscopic methods. IR spectra were performed on Bruker Tensor II FT-IR while UV spectra were recorded on Gen-5 Microplate Reader (Synergy HT). The ^{1}H NMR and ^{13}C NMR were recorded in methanol-d_4 on Bruker 600 Ultrashield NMR spectrometer measured at 600 *MHz* for ^{1}H NMR and 151 *MHz* for ^{13}C NMR. Peak multiplicities were presented in *Hz* and chemical values were shown in ppm (δ). Various chromatographic techniques have been used to purify the chemical constituents. For fractionation, liquid chromatography (VLC) was applied by using silica gel 60, 70 – 230 mesh ASTM (Merck 1.07747). Further fractionation was done by using a conventional method including VLC, CC, and *p*TLC.

Glucose uptake assay

Glucose uptake activity was performed to determine the rate of uptake of radioactively tagged 2-deoxy glucose in differentiated myoblast cells (C2C12) (Yamamoto *et al*., 2011). Initially, cells were plated in 96 well plates (black and clear bottom) at a cell density of 8×10^4 cells/mL and incubated in an incubator supplemented with 5% (v/v) CO_2 at 37 °C for overnight to allow cells to attach. Cells were then allowed for the differentiation process from myoblasts to myotubes cells. The differentiated myotube cells were treated with various concentrations of sample, from 100 – 1.562 µM for compounds and 100 – 1.562 µg/mL for crude extracts. These concentrations were used to determine the efficacy of the compounds in the stimulation of glucose uptake and compared to the positive control, that is insulin with a slight modification of the maximal concentration used previously, 200 µM (Anandharajan *et al*., 2006). The plate was read for relative fluorescence units (RFU) using a microplate reader at 465/540 nm (Tecan, USA). The fold increase of glucose uptake activity was calculated.

Cytotoxicity assay

Cytotoxicity assay was performed on C2C12 differentiated myotubes cells. Briefly, 150,000 cells/mL were plated into the 96 well plates (clear bottom). The plates were incubated in an incubator supplemented with 5% (v/v) CO_2 at 37 °C for overnight to allow cells to be attached. Cells were exposed to compounds with varying concentrations from 1000 – 1.5625 µM and 1000 – 1.562 µg/mL for crude extracts and incubated for another 24 h. The ranges of concentrations were chosen to access the highest test concentrations causing dose-dependent cytotoxic effects on cells (Swamy & Tan, 2000). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent was applied to access the metabolic activity of living cells. The assay plate was analyzed by a microplate reader (Tecan, USA) using a wavelength of 570 nm. Measurements

were performed, and the concentration required for a 50% inhibition of viability (IC_{50}) was determined.

RESULTS AND DISCUSSION

Five flavonoids namely garbanzol (**1**); one flavonol which is resokaempferol (**2**); one flavanol characterized as catechin (**3**); and two flavandiol known as mollisacacidin (**4**) and guibourtacacidin (**5**) were successfully identified from the twigs of *B. macrophylla* (Figure 1). All compounds were elucidated based on ^{1}H and ^{13}C NMR spectroscopy as well as a comparison with the spectroscopic data with values obtained from the literature.

Compound **1** was isolated as a yellow amorphous powder. The ^{13}C APT NMR spectrum displayed 13 signals representing 15 carbons. The most deshielded resonance at δ_C 192.92 is typical of a conjugated ketone signal. In addition to this, two aliphatic oxymethine carbons at δ_C 73.8 and 84.77 supported the structure of a flavanonol. Two carbon signals at δ_C 115.82 and 130.23 belong to two sets of symmetrical carbon which is typical of the *p*-hydroxybenzene ring (ring B). Additionally, the signal at δ_C 129.61, 112.58, and 103.75 are the characteristics of 7-hydroxyl substitution in ring B. The flavanonol structure as postulated by previous spectroscopic data is consistent with the signals shown in the ^{1}H NMR spectrum. Two aliphatic oxygenated methine signals resonating at δ_H 4.55 (1H, *d*, *J*=11.9 Hz) and 5.04 (1H, *d*, *J*=11.9 Hz) confirmed the *trans*-configuration of this structure. Two symmetrical *ortho*-coupled aromatic methines which resonate at 6.88 (2H, *d*, *J*=8.6 Hz) and 7.42 (2H, *d*, *J*=8.6 Hz) confirmed the presence of *p*-hydroxybenzene in ring B. As for ring A, the ABX spin system at 7.70 (*d*, *J* = 8.7 Hz, 1H), 7.44 (*d*, *J* = 8.4 Hz, 2H), 6.91 (*d*, *J* = 8.4 Hz, 2H) proved the substitution at C-7. Based on the arguments as well as comparison with reported data, compound **1** was identified as garbanzol (Nik Azmin, 2017). This compound has been isolated for the first time from the seed of *Cicer arietinum* (Wong *et al*., 1965) and showed diverse biological properties including estrogenic activity in ERE-mediated reporter gene assay (Sun *et al*., 2014) and antioxidant activity (Panthong *et al*., 2015).

Meanwhile, compound **2** was isolated as a mixture of two compounds including garbanzol (**1**), which elucidation has been described previously. The ^{13}C APT NMR spectrum displayed 26 signals representing 30 carbons which agreed with the presence of two flavonoids. The signals of the unelucidated compound were seen very similar to that of **1**, except for the C-2, C-3, and C-4. The close retention time of the compounds as well as the spectroscopic difference in ring C gave the idea that these compounds have a slightly different skeleton. This assumption was strengthened by the fact that the carbonyl signal appeared at a more upfield region, δ_C 172.04, indicating the presence of a conjugated carbonyl system. Moreover, signals for C-2 and C-3 appeared at the quarternary carbon region (δ_C 144.05 & 131.10, respectively) which corresponded to the presence of a double bond. Based on the analysis, the compound is a flavonol with the same substitution at ring A and ring B. The structure as postulated by previous spectroscopic data is consistent with the signals shown in the ^{1}H NMR spectrum. The signal which resonates at δ_H 7.01 (*d*, *J* = 2.2 Hz, 1H, H-8), 6.98 (*dd*, *J* = 8.7, 2.2 Hz, 1H. H-6), and 7.98 (*d*, *J* = 8.8 Hz, 1H, H-5) proves the substitution at C-7, while the ABX spin system at δ_H 8.17 - 7.03 is typical to the C-4' substitution in ring B. From the data elucidation, it was concluded that compound **2** is 3,7,4'-trihydroxyflavone (resokaempferol). The isolation of this compound has been reported from *Semecarpus caudata* (Dang *et al*., 2018). It has been proven to possess anticancer properties and anti-inflammatory effects (Yu *et al*., 2016).

On the other hand, compound **3** was also purified as a yellow amorphous powder. The ^{13}C NMR spectrum shows a total of 15 carbon which is characteristic of flavonoids. In this spectrum, no carbonyl signals were shown. Two methine signals appeared at d_C 81.47 (C-2) and 67.43 (C-3) which belong to ring C. A methylene carbon signal showing a long-range correlation to ring C carbon was observed at d_C 27.12 (C-4). The data listed, supported by the absence of carbonyl signals, thus described the flavan-3-ol skeletal. Furthermore, four aromatic oxyaryl carbons (δ_C 144.89-156.45), five aromatic methine carbons (δ_C 94.14-118.63), and two quaternary carbons (δ_C 99.46

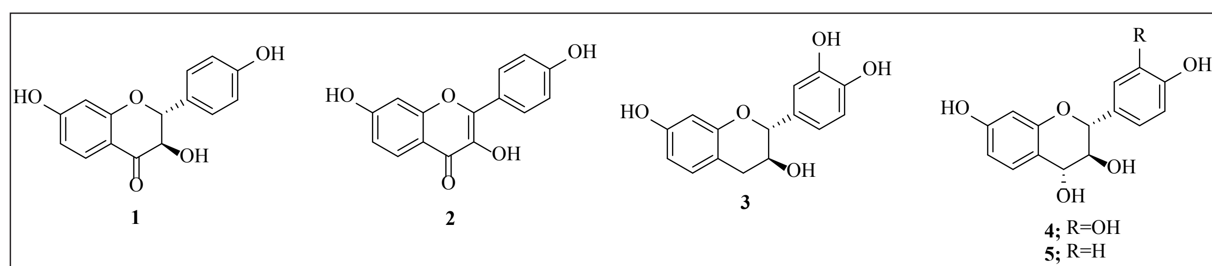

Fig. 1. Structure of compounds **1** – **5**

& 130.85) were detected in the spectrum. In the ^{1}H NMR spectrum, an ABX system was observed at d_H 6.86 (*d*, *J* = 2.0 Hz, 1H, H-2’), 6.78 (*d*, *J* = 8.1 Hz, 1H, H-5’), and 6.74 (*dd*, *J* = 8.2, 2.0 Hz, 1H, H-6’), typical for ring B with two hydroxyl substituents at C-3’ and C-4’. The *meta*-coupled doublets at d_H 5.95 (*d*, *J* = 2.0 Hz, 1H, H-6) and 5.88 (*d, J* = 2.1 Hz, 1H, H-8) belong to ring A which showed substitution at C-7. In addition, large coupling constant value (*J* = 8–10 Hz) at d_H 4.58 (*d, J* = 7.5 Hz, 1H, H-2) and 3.99 (*td, J* = 7.9, 5.4 Hz, 1H, H-3) confirmed the relative configuration as 2,3-*trans*. Thus, the structure of this compound is concluded as catechin, which is supported by the comparison with the value reported in the literature. Compound **3** has been previously isolated from the plants of the same family, including *M. indica, M. pajang,* and M. *zeylanica* (Tawaha *et al*., 2010; Al-Sheraji *et al*., 2012; Ediriweera *et al*., 2016). The same compound has been isolated from *Burkea africana* Hook. has been proven to exhibit in vitro inhibition of human telomerase activity (Eboji *et al*., 2021).

Compound **4** was isolated as a mixture of two compounds. This mixture was isolated as a yellow amorphous powder. One of the compounds was determined as compound **3**. The signals in the ^{13}C NMR spectrum belong to an unknown compound, where a total of 15 signals are shown, which is typical for a flavonoid skeletal. From this spectrum, it can be concluded that the carbonyl functional group is absent, while with two methine signals at d_C 81.33 and 73.51, the characteristics of the methine in ring C appear at a more deshielded area compared to compound **3**. Contrary to compound **3**, another methine signal at d_C 71.56 proves the presence of a substituent at C-4. This spectroscopic data summed up a flavandiol skeleton. Furthermore, the elucidation of the skeletal system is supported by the ^{1}H NMR spectrum. In this spectrum, two methine signals with large coupling constant were observed at d_H 4.67 (*d*, *J* = 8.2 Hz) and 4.59 (*d*, *J* = 7.5 Hz) which belong to H-2 and H-4, respectively. Another methine doublets resonate at d_H 3.74 (*J* = 10.0, 8.2 Hz) belongs to H-3. The splitting pattern of the doublets and large coupling constant validate the relative configuration as 2,3-*trans*-3,4-*trans*. The rest of the proton signals summed up two ABX systems which belong to rings A and B, where the substitution of the hydroxyl group occurs at C-3’, 4’, and 7. According to the spectroscopic data as well as comparison with the reported data (Drewes & Ilsley, 1968), this compound is elucidated as mollisacacidin. However, to the best of our knowledge, there are no reported activities available for this compound.

Moreover, compound **5** was also purified as a yellow amorphous powder. The ^{13}C APT NMR spectrum showed a total of 15 signals. A conjugated ketone signal can be seen at the downfield region at δ_C196.7, supported by the signals at δ_C 83.68 and 72.26, which belong to the aliphatic oxygenated methine carbons that indicate the flavanonol skeleton. According to the ^{1}H NMR spectrum, the signal of ring C is very similar to compound **4**, thus the skeleton of flavandiol is concluded. Furthermore, two sets of ABX spin system at δ_H 7.30 (*dd*, *J* = 8.5, 1.0 Hz), 6.46 (*dd*, *J* = 8.5, 2.4 Hz), 6.23 (*d*, *J* = 2.4 Hz) belong to ring A which validate the hydroxyl substitution at C-7. Moreover, two symmetrical *ortho*-coupled aromatic methines which resonate at δ_H 7.31 (*d*, *J* = 8.5 Hz, 2H) and 6.84 (*d*, *J* = 8.6 Hz) confirmed the presence of *p*-hydroxybenzene in ring B. Based on the resonance pattern, this compound is presumed to be the analog of compound **1**. Meanwhile, the signals which belong to C-2 to C-4 of ring A are very similar to flavandiol (**4**), thus the relative configuration of 2,3-*trans*-3,4-*trans* is deduced. Considering the spectroscopic evidence, this compound is elucidated as guibourtacacidin. This compound was only discovered from *Guibourtia coleosperma* (Roux & De Bruyn, 1963), followed by *Acacia mearnsii* (Tindale and Roux, 1969) of which the elucidation was done by classic analysis including 2D chromatograms, chemical tests for functional group determination, elemental analysis, and comparative study. According to Nel *et al*. (1999), leucoguibourtinidins and their derivatives with their 4’,7-dihydroxy phenolic functionality are relatively rare. Thus, this might be the first report describing the NMR spectroscopic data of this compound, and no reported activities are available for this compound. Table 1 shows the summary of NMR data obtained for isolated compounds.

Type 2 Diabetes mellitus (T2DM) is commonly associated with obesity, genetics, and physical inactivity, and it might cause other complications such as high blood pressure, heart disease, and kidney failure. One of the strategies for T2DM patients to maintain proper blood glucose levels is by enhancing the glucose uptake of organs or tissues. In the present study, the 2-deoxyglucose uptake assay was designed to evaluate the insulin-like and insulin-sensitizing activity of isolated compounds from *Bouea macrophylla* Griff. The myoblast cell line, C2C12, has been utilized extensively in vitro as an examination model for understanding metabolic disease progression (Wong *et al*., 2020). EA crude extract (BMSEA) which was chosen for the isolation of flavonoids and two compounds with the sufficient amount, compounds **1** and **3,** were tested for glucose uptake activity. BMSEA showed an increase in glucose uptake activity and the highest uptake was produced at a concentration of 1.562 µg/mL with 0.96-fold (Figure 2). Additionally, compound **1** (garbanzol) and **3** (catechin) exhibited an interesting activity in glucose uptake assessment when compared with rosiglitazone, standard drug and insulin that act as a

positive control. Garbanzol significantly increased glucose transport into C2C12 myotubes, in a dose-dependent manner from 50 µM to 3.125 µM with the maximal glucose uptake of 0.986 ± 0.04-fold increase at 3.125 µM. As for catechin, it showed a decrease in a dose-dependent manner with minor glucose uptake activities of 0.971 ± 0.08-fold increase at 100 µM. In comparison, a dose of 6.25 µM rosiglitazone yielded a maximal effect of 0.836 ± 0.009-fold increase in this cellular model yet, showed both compounds possess a better activity than a standard drug (Figure 3). No toxicity effect was noticed for both compounds when tested on differentiated myotubes C2C12 cell line, with IC_{50} value of more than 1000 µM (IC_{50} > 1000 µM). Table 2 represents the fold increase in glucose uptake activity and cytotoxicity activity.

Table 1. NMR data of isolated compounds

Position	**1**		**2**		**3**		**4**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.02, *d*	84.77		131.10	4.58, *d*	81.47	4.67, *d*	81.33	4.68, *d*	81.18
3	4.53, *d*	73.83		129.34	3.99, *td*	67.43	3.74, *dd*	73.51	3.76, *dd*	73.47
4		192.92		172.04	2.87, *dd* 2.52, *dd*	27.12	4.59, *d*	71.56		71.63
5	7.70, *d*	129.61	7.98, *d*	126.36		156.18	7.30, *d*	128.24	7.30, *dd*	128.27
6	6.59, *dd*	112.58	6.98, *dd*	115.29	5.95, *d*	94.94	6.46, *dd*	108.58	6.46, *dd*	108.62
7		168.02		164.18		156.45		157.68		157.65
8	6.37, *d*	103.75	7.01, *d*	102.19	5.88, *d*	94.14	6.23, *d*	101.79	6.23, *d*	101.80
9		164.76		144.05		155.53		155.06		157.29
10		111.95		116.44		99.46		116.14		116.19
1'		129.48		123.00		130.85		130.87		129.18
2'	7.44, *d*	130.23	8.17, *d*	126.36	6.86, *d*	113.89	6.94, *brs*	113.90	7.31, *d*	128.97
3'	6.91, *d*	115.82	7.03, *d*	115.40		144.84		144.87	6.84, *d*	114.68
4'		158.77		157.85		144.87		145.27		155.08
5'	6.91, *d*	115.82	7.03, *d*	115.40	6.78, *d*	114.71	6.81, overlapped	114.64	6.84, *d*	114.68
6'	7.44, *d*	130.23	8.17, *d*	126.36	6.74, *dd*	118.65	6.81, overlapped	119.58	7.31, *d*	128.97

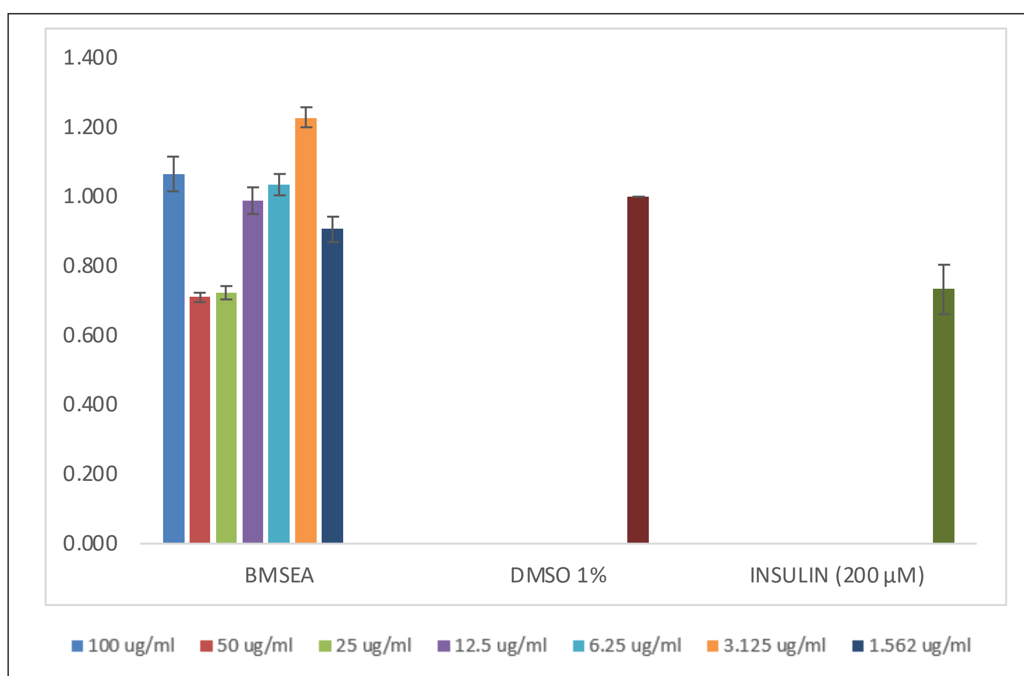

Fig. 2. Glucose uptake assay of crude extract (BMSEA) on the insulin-resistant C2C12 cell line. Glucose uptake activity in the untreated control (1% (v/v) DMSO) was assigned as 1-fold.

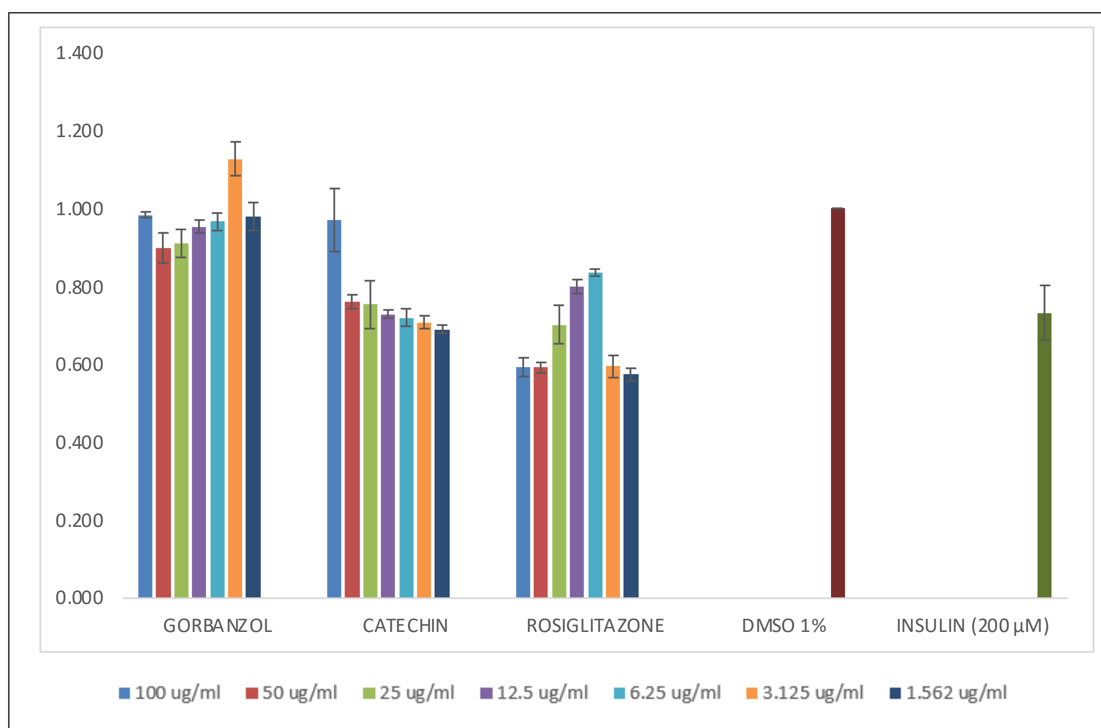

Fig. 3. Glucose uptake assay of garbanzol (compound **1**), catechin (compound **3**), Rosiglitazone (standard drug), and insulin (positive control) on insulin-resistant C2C12 cells. Glucose uptake activity in the untreated control (1% (v/v) DMSO) was assigned as 1-fold.

Table 2. The fold increase in glucose uptake activity and cytotoxicity activity

Extract/Compound	Glucose Uptake Rate	Cytotoxicity Activity
BMSEA	0.96 at 1.56 µg/mL	IC_{50} = 437.27 µg/mL
Garbanzol (Comp. 1)	0.80 at 3.13 µg/mL	IC_{50} > 100 µM
Catechin (Comp. 3)	0.54 at 1.56 µg/mL	IC_{50} > 100 µM
Rosiglitazone	0.53 at 6.25 µg/mL	IC_{50} > 100 µM

CONCLUSION

Five flavonoids namely garbanzol (**1**), resokaempferol (**2**), catechin (**3**), mollisacacidin (**4**), and guibourtacacidin (**5**) were successfully isolated, all of which were identified for the first time from *B. Macrophylla*. The results of the glucose uptake experiment indicated that the extract and compounds showed activity on the glucose uptake rate of the insulin-resistant C2C12 cell line as compared to the standard with no toxicity effect when tested on MTT assay. As previous reports found that these types of phenolic compounds displayed diverse pharmacological properties, further detailed investigation of their biological activities is recommended.

ACKNOWLEDGEMENTS

The authors would like to thank The Ministry of Higher Education, Malaysia for the financial support under the MyPhD Scholarship, GIP grant 600-RMC/GIP 5/3 (033/2021) for financing this research project and Universiti Teknologi MARA (UiTM) for providing laboratory and facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Al-Sheraji, S.H., Ismail, A., Manap, M.Y., Mustafa, S. & Yusof, R.M. 2012. Fermentation and non-digestibility of *Mangifera pajang* fibrous pulp and its polysaccharides. *Journal of Functional Foods*, **4(4)**: 933–940. https://doi.org/10.1016/j.jff.2012.07.001

Anandharajan, R., Jaiganesh, S., Shankernarayanan N.P., Viswakarma, R.A. & Balakrishnan, A. 2006. In vitro glucose uptake activity of *Aegles marmelos* and *Syzygium cumini* by activation

of Glut-4, PI3 kinase and PPARgamma in L6 myotubes. *Phytomedicine*, **13**: 434-441. https://doi.org/10.1016/j.phymed.2005.03.008

Dang, P.H., Nguyen, T.T., Le, T.H., Nguyen, H.X., Nguyen, M.T.T. & Nguyen N.T. 2018. A new bischromanone from the stems of *Semecarpus caudata*. *Natural Product Research*, **32(15)**: 1745–1750. https://doi.org/10.1080/14786419.2017.1399391

Drewes, S.E. & Ilsley, A.H. 1969. Isomeric leucofisetinidins from *Acacia mearnsii*. *Phytochemistry*, **8**:1039-1042. https://doi.org/10.1016/S0031-9422(00)86352-9

Eboji, O.K., Borges, G., Harrington, L., Lin, W., Sofidiya, M.O. & Sowemimo, A.A. 2021. Catechin from *Burkea africana* Hook. exhibits in vitro inhibition of human telomerase activity. *Natural Product Research*, **35(24)**: 6175-6179. https://doi.org/10.1080/14786419.2020.1831497

Ediriweera, M.K., Tennekoon, K.H., Adhikari, A., Samarakoon, S.R., Thabrew, I. & de Silva, E.D. 2016. New halogenated constituents from *Mangifera zeylanica* Hook. F. and their potential anti-cancer effects in breast and ovarian cancer cells. *Journal of Ethnopharmacology*, **189**: 165–174. https://doi.org/10.1016/j.jep.2016.05.047

Nel, R.J.J., Mthembu, M., Coetzeea, J., van Rensburg, H., Malan, E. & Ferreira, D. 1999. The novel flavan-3-ol, (2R,3S)-guibourtinidol and its diastereomers. *Phytochemistry*, **52(6)**: 1153–1158. https://doi.org/10.1016/S0031-9422(99)00348-9

Ng, F.S.P. 1989. Tree Flora of Malaya. Volume 4. Longman Malaysia.

Nik Azmin, N.F. 2017. Phytochemical studies of *Gnetum microcarpum, Gnetum cuspidatum, Cynometra cauliflora, Bouea oppositifolia* and their biological activities (Ph.D). Universiti Teknologi MARA.

Panthong, K., Sompong, R., Rukachaisirikul, V., Towatana N.H., Voravuthikunchai, S.P. & Saising, J. 2015. Two new triterpenes and a new coumaroyl glucoside from the twigs of *Mangifera foetida* Lour. *Phytochemistry Letters*, **11**: 43–48. https://doi.org/10.1016/j.phytol.2014.11.007

Rana, A., Sharma, E., Rawat, K., Sharma, R., Verma, S., Padwad, Y. & Gulati, A. 2016. Screening and purification of catechins from underutilized tea plant parts and their bioactivity studies. *Journal of Food Science and Technology*, **53(11)**: 4023–4032. https://doi.org/10.1007/s13197-016-2406-6

Roux, D.G. & Bruyn, G.C.D. 1963. Isolation of 4',7-dieiydroxyflavan-3,4-diol from *Guibourtia coleosperma*. *Toxic Substances in Crop Plants*, **87(1957)**: 439–444. https://doi.org/10.1533/9781845698454.180

Sun, S., Choi, Y.H., Na, C.S., Lee, D., Yoo, H.H., Hong C.Y., Ahn, B Y. & Dong, M.S. 2014. Estrogenic activity of a *Rhus verniciflua* extract and its major components. *Journal of Functional Food*, **11**: 250-260. https://doi.org/10.1016/j.jff.2014.09.014

Tawaha, K., Sadi, R., Qa'dan, F., Matalka, K.Z. & Nahrstedt A. 2010. A bioactive prodelphinidin from *Mangifera indica* leaf extract. *Zeitschrift fur Naturforschung - Section C Journal of Biosciences*, **65C(5–6)**: 322–326. https://doi.org/10.1515/znc-2010-5-603

Tindale, M.D. & Roux, D.G. 1969. A phytochemical survey of the Australian species of *Acacia*. *Phytochemistry*, **8(9)**: 1713–1727. https://doi.org/10.1016/S0031-9422(00)85959-2

Wong, E., Mortimer, I.P. & Geissman, T.A. 1965. Flavonoid constituents of *Cicer arietinum*. *Phytochemistry*, **4(1)**: 89-95. https://doi.org/10.1016/S0031-9422(00)86150-6

Wong, C.Y., Al-Salami, H. & Dass, C.R. 2020. C2C12 cell model: its role in understanding of insulin resistance at the molecular level and pharmaceutical development at the preclinical stage. *Journal of Pharmacy and Pharmacology*, **72(12)**: 1667-1693. https://doi.org/10.1111/jphp.13359

Yamamoto, Y., Ito, A., Fujita, H., Nagamori, E., Kawabe, Y. & Kamihira, M. 2011. Functional evaluation of artificial skeletal muscle tissue constructs fabricated by a magnetic force-based tissue engineering technique. *Tissue Engineering: Part A*, **17(1-2)**: 107-114. https://doi.org/10.1089/ten.tea.2010.0312

Yu, Q., Zeng, K.W., Ma, X.L., Song F.J., Jiang Y., Tu, P.F. & Wang, X.M. 2016. Resokaempferol-mediated anti-inflammatory effects on activated macrophages via the inhibition of JAK2/STAT3, NF-κB and JNK/p38 MAPK signaling pathways. *International Immunopharmacology*, **38**: 104–114. https://doi.org/10.1016/j.intimp.2016.05.010