Jordan Journal of Pharmaceutical Sciences, Volume 12, No. 1, 2019

In Vitro Pharmacological Evaluation of the Leaves and Stem Bark Extracts of *Beilschmiedia penangiana* Gamble

Wan Mohd Nuzul Hakimi Wan Salleh[1]*, Farediah Ahmad[2], Saripah Salbiah Syed Abdul Azziz[1], Mohamad Syahrizal Ahmad[1]

[1] Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris (UPSI), 35900 Tanjong Malim, Perak, Malaysia

[2.] Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM), 81310 Johor Bahru, Johor, Malaysia.

ABSTRACT

The leaves and stem bark extracts of *B. penangiana* were screened to evaluate their antioxidant, antimicrobial, tyrosinase, lipoxygenase, and acetylcholinesterase activities. Evaluation of antioxidant activities were tested against DPPH, ABTS, and total phenolic content (TPC) assays. The antimicrobial activity were investigated by microdilution method for determination of MIC, MBC, and MFC. Tyrosinase and lipoxygenase inhibitory activities were analysed using mushroom tyrosinase and lipoxygenase enzymes, respectively. In addition, the acetylcholinesterase activity was tested using cholinesterase enzyme. The methanol extract of stem bark showed the highest phenolic content (176.8 mg GAE/g), DPPH free radical scavenging (IC_{50} 95.5 µg/mL), ABTS (IC_{50} 124.8 µg/mL), and lipoxygenase inhibitory activity (I: 46.3%). All extracts showed from moderate to weak activity with MIC/MBC/MFC values in the ranged of 62.5-1000 µg/mL. The ethyl acetate stem bark extract revealed the highest percentage inhibition on tyrosinase activity (I: 83.2%), while for acetylcholinesterase activity showed by the methanol leaves extract with percentage inhibition of 69.4%. The results revealed that the extracts of *B. penangiana* possessed significant activities on various biological studies. Further investigations will be focussing on isolation of the secondary metabolites responsible for the bioactivities.

Keywords: Beilschmiedia, Lauraceae, Antioxidant, Antimicrobial, Antityrosinase; Lipoxygenase, Acetylcholinesterase.

1. INTRODUCTION

The genus *Beilschmiedia* comprises about 250 plant species widely distributed in tropical Asia and Africa. These plants produce several classes of natural products including endiandric acid derivatives, essential oils, lignans, neolignans, flavonoids, lactones and alkaloids. Few of these compounds were reported to exhibit antibacterial, antimalarial, anti-inflammatory, anti-oxidant, anti-α-glucosidase, anti-leishmanial, anti-fungal, anti-cholinesterase and antituberculosis activities.[1-3] In Africa, some of the species from this genus were used as folk medicines in treating uterine tumors, rheumatism, and pulmonary diseases.[4] Several biological activities of the extracts of *Beilschmiedia* species also had been reported. Previous reports on the extracts of *B. obscura*, *B. cinnamomea*, *B. tovarensis*, *B. acuta* and *B. cinnamomea* had shown antibacterial and antifungal

* wmnhakimi@fsmt.upsi.edu.my.

Received on 27/1/2018 and Accepted for Publication on 8/8/2018.

properties. The extract of *B. erythrophloia* was found as radical scavenger, while *B. erythrophloia* and *B. tsangii* displayed potent inhibition on anti-inflammatory activity.[5-9]

Beilschmiedia penangiana is commonly known as '*medang ayer*' in Malaysia. It grows in lowland or hill forests.[10] The chemical compositions and biological activities of the leaf and bark oils from *B. penangiana* had been reported recently. The essential oils showed significant β-carotene/linoleic acid bleaching (leaf 67.2%; bark 112.4%) and DPPH radical-scavenging (IC_{50}: leaf 92.4 μg/mL; bark 88.3 μg/mL) activities, and strong antifungal property against *Candida glabra* (MIC 31.3 μg/mL). Besides, the essential oils also displayed significant inhibition of tyrosinase (leaf 80.6%; bark 82.5%), acetylcholinesterase (leaf 51.8%; bark 47.1%), and lipoxygenase (leaf 32.0%; bark 45.8%) activities.[11] In continuation of our bioactivity studies on this species, herein we report the antioxidant, antimicrobial, antityrosinase, lipoxygenase, and acetylcholinesterase activities from the leaves and stem bark extracts of *B. penangiana*.

MATERIALS AND METHODS

Plant material

B. penangiana (Voucher no. SK2572/14) was collected from Kluang, Johor in October 2014. The species were identified by Dr. Shamsul Khamis (UPM) and the voucher specimens were deposited at the Herbarium of Institute of Bioscience, Universiti Putra Malaysia.

Extraction method

The dried and powdered leaves (250 g) and stem bark (200 g) were extracted with *n*-hexane (Hex), ethyl acetate (EtOAc) and methanol (MeOH), sequentially. The extracts were filtered and the solvent was removed under vacuum using a rotary evaporator (Eyela, Germany) to obtain the Hex, EtOAc and MeOH extracts. Percentage yields (w/w) of the extracts obtained are shown in Table (1).

Table (1)
Percentage of yield and antioxidant activities of B. penangiana extracts

Parts	Extracts	Yield	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)	TPC (mg GAE/g)
Leaves	Hex	1.82 g, 0.91 %	105.0±0.3	157.1±0.1	101.5 ± 0.4
	EtOAc	4.02 g, 2.01 %	200.5±0.1	215.9±0.2	66.8 ± 0.1
	MeOH	7.76 g, 3.88 %	177.0±0.3	141.0±0.3	59.6 ± 0.5
Stem Bark	Hex	3.54 g, 1.77%	143.7±0.1	276.3±0.3	47.7 ± 0.2
	EtOAc	5.35 g, 2.67 %	155.1±0.1	228.1±0.3	46.3 ± 0.2
	MeOH	8.82 g, 4.41 %	95.5±0.3	124.8±0.1	176.8 ± 0.3
BHT			18.5	52.2	ND

Data represent as mean ± SD of three independent experiments; ND – not determined; TPC - Total phenolic content; GAE – gallic acid equivalent; BHT - Butylated hydroxytoluene

Solvents and chemicals

Analytical grade *n*-hexane, ethyl acetate and methanol used for extraction were purchased from Merck (Germany). Antioxidants: 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich (Germany). Analytical grade methanol, ethanol and dimethylsulfoxide (DMSO), HPLC grade chloroform, and Folin–Ciocalteu's reagent were purchased from Merck (Germany). Antimicrobial: Nutrient agar (NA), nutrient broth (NB), sabouraud dextrose agar (SDA), sabouraud dextrose broth (SDB), streptomycin sulphate

and nystatin were purchased from Oxoid (Italy). All tested microorganisms were purchased from Mutiara Scientific (Malaysia). Tyrosinase: Mushroom tyrosinase enzyme (EC1.14.18.1), kojic acid and L-dopa were purchased from Sigma-Aldrich (Germany). Lipoxygenase: Lipoxygenase inhibitor screening assay kit (Item No. 760700 Cayman Chemicals Co) was purchased from i-DNA Biotechnology (M) Sdn. Bhd. (Malaysia). Acetylcholinesterase: AChE enzyme (Type-VI-S, EC3.1.1.7), acetylthiocholine iodide, 5,5′-dithio-bis (2-nitrobenzoic) acid (DTNB), and galantamine were purchased from Sigma-Aldrich (Germany).

Antioxidant activity

DPPH free radical scavenging assay

The free radical scavenging activity was measured by the DPPH method with minor modifications.[12] Briefly, 0.1 mM DPPH (1 mL) dissolved in EtOH was added to an EtOH solution (3 mL) of the tested samples and standard (BHT) at different concentrations (200, 150, 100, 50, 25 µg/mL). An equal volume of EtOH was added in the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance at 517 nm was measured with a UV–vis spectrophotometer. The percentage of inhibitions (I%) of DPPH radical were calculated as follow:

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

where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test extracts) and A_{sample} is the absorbance values of the test extracts/standard. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests and IC_{50} values were reported as means ± SD of triplicates.

ABTS radical scavenging

The antioxidant capacity was estimated by ABTS radical scavenging activity following the previously reported method.[13] Briefly, ABTS was obtained by reacting 14 mM ABTS stock solution (5 mL) with 4.9 mM potassium persulfate (5 mL) and the mixture was left to stand in the dark at room temperature for 12–16 h before use. The ABTS solution (stable for 2 days) was diluted with distilled water to an absorbance at 734 nm of 0.70 ± 0.02 by UV-vis spectrophotometer. After the addition of 15 µL of extracts (concentration of 200, 150, 100, 50, 25 µg/mL) to 285 µL of diluted ABTS solution, the absorbance was measured after 30 min. The same procedure was repeated for the standard gallic acid solutions. The percentage inhibition (I%) of ABTS radical-scavenging activity of the extracts was expressed as:

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

where $A_{control}$ is the absorbance of the blank control (ABTS solution without extracts) and A_{sample} is the absorbance of the extracts. All tests were carried out in triplicate.

Total phenolic content (TPC) assay

TPC of the extracts was measured using gallic acid equivalent with minor modifications.[12] A sample of stock solution (1.0 mg/mL) was diluted in MeOH to final concentrations of 1000 µg/mL. A 0.1 mL aliquot of sample was pipetted into a test tube containing 0.9 mL of methanol, then 0.05 mL Folin-Ciocalteu's reagent was added, and the flask was thoroughly shaken. After 3min, 0.5 mL of 5% Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h with intermittent shaking. Then, 2.5 mL of methanol was added and left to stand in the dark for 1 h. The absorbance measurements were recorded at 765 nm. The same procedure was repeated for the standard gallic acid solutions. The concentration of total phenolic compounds in the extracts was expressed as mg of gallic acid equivalent per gram of sample (mg GAE/g). Tests were performed in triplicates.

Antimicrobial activity

Microbial strains

Nine microorganisms; three Gram-positive bacteria; *Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (ATCC29737) and *Enterococcus faecalis* (ATCC19433), three Gram-negative bacteria; *Pseudomonas aeruginosa*

(ATCC9027), *Escherichia coli* (ATCC10536) and *Klebsiella pneumoniae* (ATCC13883) and three fungal/yeast *Aspergillus niger* (ATCC16888), *Candida glabrata* (ATCC2001) and *Saccharomyces cerevisiae* (ATCC7754) were used. The strains were grown on NA for the bacteria and SDA for fungal/yeast. For the activity tests, NB for bacteria and SDB for fungal/yeast strains were used.

Microdilution method

Antimicrobial test was carried out by MIC (minimum inhibitory concentration), MBC (minimum bactericidal concentration) and MFC (minimum fungicidal concentration) by broth micro dilution method using 96-well microplates.[12] The inocula of the microbial strains were prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts (1 mg) were dissolved in DMSO (1 mL) to get 1000 µg/mL stock solution. A number of wells (A–H) were reserved in each plate for positive and negative controls. Sterile broth (100 µL) was added to the wells from row B to H. The stock solutions of samples (100 µL) were added to the wells at row A and B. Then, the mixture of samples and sterile broth (100 µL) at row B 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, 15.6 and 7.8 µg/mL). The inoculum (100 µL) was added to each well. The final volumes in each well were 200 µL. Streptomycin sulphate for bacteria and nystatin for fungal/yeast were used as positive controls. Plates were incubated at 37°C for 24 h. Microbial growth was indicated by the presence of turbidity and a pellet at the bottom of the well. Samples from the MIC study which did not show any growth of bacteria were removed from each well (10 mL) and then subculture on the surface of the freshly prepared nutrient agar in disposable Petri dishes (50mm×15 mm). Then, the Petri dishes were inverted and incubated for 16-20 h at 37°C. After 16-20 h, the number of surviving organisms was determined.

Tyrosinase inhibitory activity

Tyrosinase inhibitiory assay was carried out following the standard method with slight modifications.[14] Briefly, the extracts and kojic acid were dissolved in DMSO prepared as 1 mg/mL. The reaction was carried out using 96-well microplate and microplate reader (Epoch Micro-Volume Spectrophotometer, USA) was used to measure the absorbance at 475 nm. 40 µL of extracts dissolved in DMSO with 80 µL of phosphate buffer (pH 6.8), 40 µL of tyrosinase enzyme and 40 µL of L-dopa were put in each well. Each sample was accompanied by a blank that had all the components except for L-dopa. Kojic acid was used as reference standard inhibitor for comparison. The percentage of tyrosinase inhibition (I%) was calculated as follows:

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

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the extracts/reference. Analyses were expressed as means ± SD of triplicates.

Lipoxygenase inhibitory activity

Lipoxygenase (LOX) inhibition was determined using an enzyme immuno assay (EIA) kit (Catalog No. 760700, Cayman Chemical, USA) according to the manufacturer's instructions. The Cayman Chemical lipoxygenase inhibitor screening assay detects and measures the hydroperoxides produced in the lipoxygenation reaction using a purified lipoxygenase. Stock solutions of the extracts were dissolved in a minimum volume of DMSO and were diluted using the supplied buffer solution (0.1 M, Tris–HCl, pH 7.4). To a 90 µL solution of 5-LOX enzyme in 0.1 M Tris–HCl, and pH 7.4 buffer, 10 µL of various concentrations of test samples (final volume of 210 µL) were added and the lipoxygenase reaction was initiated by the addition of 10 µL (100 µM) of arachidonic acid. After maintaining the 96-well plates on a shaker for 5 min, 100 µL of chromogen was added and the plate was retained on a shaker for 5 min. The lipoxygenase activity was determined after measuring absorbance at a wavelength of 500 nm. The percentage inhibition (I%) of the extracts was calculated as follows:

$$I\% = [\ A_{initial\ activity} - A_{inhibitor} / A_{initial\ activity}\ ] \times 100$$

where $A_{initial\ activity}$ is the absorbance of 100% initial

activity wells without sample and $A_{inhibitor}$ is the absorbance of extracts/reference. All tests were carried out in triplicate and expressed as means ± SD.

Acetylcholinesterase (AChE) activity

AChE inhibitory activity of the extracts was measured by slightly modifying the spectrophotometric method.[14] Electric eel AChE was used, while acetylthiocholine iodide (AChI) was employed as the substrate of the reaction. DTNB acid was used for the measurement of the AChE activity. Briefly, 140 µL of sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of test compounds and 20 µL of AChE solution were added by multichannel automatic pipette in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of 10 µL of AChI. Hydrolysis of AChI was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (Epoch Micro-Volume Spectrophotometer, USA). Percentage of inhibition (I%) of AChE was determined by comparison of rates of reaction of samples relative to blank sample (EtOH in phosphate buffer pH = 8) using the formula:

$I\% = [E – S / E] \times 100;$

where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Galantamine was used as the positive controls.

Statistical analysis

Data obtained from the biological activities are expressed as mean ±SD of triplicates. The statistical analyses were carried out by employing one-way ANOVA ($p < 0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

RESULTS AND DISCUSSION

Study of antioxidant efficiency in extracts using ABTS and DPPH radical scavenging assay is an important in *in vitro* analysis. Both methods apply decolorization assays to identify the existence of antioxidant which annul the development of the ABTS and DPPH radicals.[15] The antioxidant activity of *B. penangiana* extracts are summarised in Table (1). For DPPH and ABTS assays, the highest activity was showed by the MeOH stem bark extract with IC_{50} value of 95.5 and 124.8 µg/mL, respectively. In most of the assays to determine the antioxidant properties, the ABTS activity was strongly correlated with DPPH because both methods are responsible for the same chemical property of H^- or electron-donation to the antioxidant.[16-17] In addition, the MeOH stem bark extract also found to have the highest TPC (176.8 mg GA/g). Phenolic compounds are known as plants antioxidant agents due to their capability as reducing agents, hydrogen donors, and singlet oxygen scavengers.[18] Phenolic compounds such as flavonoids are noted as antioxidant agents. The plant extracts also contained some flavonoids as reported previously from *Beilschmiedia* species such as *B. zenkeri*, *B. tovarensis* and *B. miersii*.[19-20]

Table (2) shows the MIC, MBC, and MFC results obtained from the leaves and stem bark extracts of *B. penangiana* against nine microorganisms. The MeOH stem bark extract showed the best activity against *Enterococcus faecalis* with MIC value of 62.5 µg/mL. However, other extracts displayed moderate to weak activity with MIC/MBC/MFC ranging from 125-1000 µg/mL towards three Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Enterococcus faecalis*) and three Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*). It is often reported that Gram-negative bacteria are more resistant to the plant extracts, since the hydrophilic cell wall structure of Gram-negative bacteria is constituted essentially of a lipopolysaccharide that blocks the penetration of hydrophobic oil and avoids the accumulation of extracts in the target cell membrane. This is the reason that Gram-positive bacteria were found to be more sensitive to the extracts of *B. penangiana* than Gram-negative ones.[21] For antifungal activity, the best activity was showed by the MeOH stem bark extract with MFC value of 125 µg/mL against the fungal strains

(*Aspergillus niger*, *Candida glabrata*, and *Saccharomyces cerevisiae*). Some isolated compounds from the genus *Beilschmiedia* have been reported to have antimicrobial activities. Compounds with antifungal and antibacterial activities have been isolated from other species of *Beilschmiedia* which are beilschmiedic acid A, beilschmiedic acid K, beilschmiedic acid L, cryptobeilic acid A, and cryptobeilic acid B.[22-23]

Table (2)
Antimicrobial activity of *B. penangiana* extracts

Extracts/Microbes		Gram-positive bacteria						Gram-negative bacteria						Fungal strains							
		BS		SA		EF		PA		EC		KP		AN		CG		SC	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC	MIC	MFC
Leaves	Hex	1000	1000	500	500	125	250	250	500	125	250	500	1000	250	250	250	250	250	250
	EtOAc	250	250	500	500	125	250	250	500	250	500	500	1000	250	250	125	250	250	250
	MeOH	125	250	125	250	62.5	125	125	250	250	500	250	500	125	125	125	125	125	125
Stem Bark	Hex	1000	1000	1000	1000	125	250	250	250	125	250	250	500	250	250	1000	1000	250	250
	EtOAc	500	500	500	1000	125	250	500	500	250	500	250	500	500	1000	250	250	125	125
	MeOH	500	1000	500	500	125	250	500	500	250	500	250	500	250	250	250	250	250	250
SS		7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	ND	ND	ND	ND	ND	ND
NYS		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.8	7.8	7.8	7.8	7.8	7.8

Results of MIC/MBC/MFC in µg/mL; SS – Streptomycin sulphate; NYS – nystatin; BS – *Bacillus subtilis*; SA – *Staphylococcus aureus*; EF – *Enterococcus faecalis*; PA – *Pseudomonas aeruginosa*; EC – *Escherichia coli*; KP – *Klebsiella pneumonia*; AN– *Aspergillus niger*; CG – *Candida glabrata*; SC– *Saccharomyces cerevisiae*; ND – not determined.

Table (3)
Tyrosinase, lipoxygenase, and acetylcholinesterase inhibitory activities of *B. penangiana* extracts

Parts	Extracts	Tyrosinase (I%)	Lipoxygenase (I%)	Acetylcholinesterase (I%)
Leaves	Hex	67.6 ± 0.3	39.8 ± 0.2	65.3 ± 0.5
	EtOAc	80.4 ± 0.1	45.8 ± 0.2	69.3 ± 0.5
	MeOH	81.9 ± 0.2	32.4 ± 0.2	69.7 ± 0.5
Stem Bark	Hex	74.3 ± 0.1	24.2 ± 0.3	53.3 ± 0.8
	EtOAc	83.2 ± 0.2	41.9 ± 0.3	32.2 ± 1.5
	MeOH	81.2 ± 0.2	46.3 ± 0.4	20.2 ± 1.2
Kojic acid		97.1 ± 0.1	ND	ND
Quercetin		ND	89.1 ± 0.2	ND
Acarbose		ND	ND	97.7 ± 0.4

Data presented as mean ± SD of three independent experiments; ND – not determined.

Table (3) summarizes the results of tyrosinase, lipoxygenase, and acetylcholinesterase inhibitory activities at the concentration of 1 mg/mL. Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions, such as food browning and melanisation of human skin. Therefore, these agents have good commercial potential in both food processing and cosmetic industries. All extracts showed good inhibitory activity with the percentage inhibition in the range of 67.6 - 83.2%. The EtOAc stem bark extract displayed the highest tyrosinase inhibition activity which gave 83.2% inhibition. The inhibition of tyrosinase might depend on the hydroxyl groups of phenolic compounds of the mushroom extracts that could form a hydrogen bond to the active site of the enzyme, leading to a lower enzymatic activity. Some tyrosinase inhibitors act through hydroxyl groups that bind to the active site on tyrosinase enzyme, resulting in steric hindrance or change in conformation.[24]

Lipoxygenases (LOX) are the key enzymes in the biosynthesis of leukotrienes that play an important role in several inflammatory diseases. Inhibition of LOX may influence the inflammation processes and thus be of interest for modulation of the lipoxygenase pathway. Therefore, inhibitors of oxidative stress and LOX have been considered as therapeutically useful in the treatment of many related diseases such as liver disease, arthritis and cancer.[25] In lipoxygenase inhibitory activity, a standard quercetin, inhibited lipoxygenase activity by 89.1% at 1 mg/mL. All extract exhibited weak to moderate lipoxygenase inhibitory activity ranging from 24.2-46.3%. The highest activity showed by the MeOH

stem bark extract with percentage inhibition of 46.3%. It is noteworthy that, the MeOH stem bark extract had the highest TPC with good antioxidant activity, a finding which is consistent with a previous study which found a relationship between the anti-inflammatory activity and the presence of polyphenols. Antioxidants are also known to inhibit plant lipoxygenases.[26]

In acetylcholinesterase (AChE) activity, all extracts were found to have weak to moderate activity, in the range of 20.2- 69.7%, comparable to galantamine, 97.7%. The MeOH leaves extract exerted the highest AChE activity with inhibition of 69.7%, followed by their EtOAc (69.3%) and Hex (65.3%) extract. Inhibition of AChE, the key enzyme in the breakdown of acetylcholine, is considered as one of the treatment strategies against several neurological disorders such as Alzheimer disease. Since the strongest synthetic or natural product driven AChE inhibitors are known to contain nitrogen, the promising activity of *B. penangiana* extracts could be due to their alkaloidal contents.[27-30]

CONCLUSION

Based on these results, it is conceive to conclude that the leaves and stem bark extracts of *B. penangiana* had significant level of antioxidant, antimicrobial, antityrosinase, lipoxygenase, and acetylcholinesterase activities. Investigations are in progress to determine the secondary metabolites of these extracts. Evaluation of possible synergism among chemical components for their biological activities also will be carried out in order to study in-depth of their pharmacology safety to capitulate potential new therapeutic drugs or drug leads.

Acknowledgements

The authors would like to thank the University Research Grant (GPU) for financial support under vote 2017-0144-101-01 and the Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris for research facilities.

REFERENCES

(1) Salleh W. M. N. H. W., Ahmad F., Khong H. Y., Zulkifli R. M. A review on phytochemistry and pharmacology of the genus *Beilschmiedia* (Lauraceae). *Trop. J. Pharm. Res.* 2015; 14 (11): 2139-2150.

(2) Salleh W. M. N. H. W., Ahmad F., Khong H. Y., Zulkifli R. M. A review of endiandric acid analogues. *Int. J. Pharmacogn. Phytochem. Res.* 2015; 7 (4): 844-856.

(3) Salleh W. M. N. H. W., Ahmad F., Khong H. Y., Zulkifli R. M. Essential oil composition of Malaysian Lauraceae: A mini review. *Pharm. Sci*. 2016; 22 (1): 60-67.

(4) Iwu M. M. Handbook of African medicinal plants, 1st ed. Boca Raton: CRC Press 1993.

(5) Suarez L. E. C, Vargas O. E. B. New chalcones from *Beilschmiedia tovarensis*. *Rev. Colomb. Quim.* 2005; 34: 35-41.

(6) Fankam A. G., Kuete V., Voukeng I. K., Kuiate J. R., Pages J. M. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug resistant phenotypes. *BMC Complement. Altern. Med.* 2011; 11: 104-114.

(7) Fankam A. G., Kuiate J. R., Kuete V. Antibacterial activities of *Beilschmiedia obscura* and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes. *BMC Complement. Altern. Med*. 2014; 14: 241-250.

(8) Lin C. T., Chu F. H., Tseng Y. H., Tsai J. B., Chang S. T., Wang S. Y. Bioactivity investigation of Lauraceae trees grown in Taiwan. *Pharm. Biol*. 2007; 45: 638-644.

(9) Huang Y. T., Chang H. S., Wang G. J., Cheng M. J., Chen C. H., Yang Y. J., Chen I. S. Anti-inflammatory endiandric acid analogues from the roots of *Beilschmiedia tsangii*. *J. Nat. Prod.* 2011; 74: 1875-1880.

(10) Kok R. P. J. A revision of *Beilschmiedia* (Lauraceae) of Peninsular Malaysia. *Blumea* 2016; 61: 147-164.

(11) Salleh W. M. N. H. W., Ahmad F., Khong H. Y., Zulkifli R. M. Comparative study of the essential oils of three *Beilschmiedia* species and their biological activities. *Int. J. Food Sci. Tech.* 2016; 51: 240-249.

(12) Salleh W. M. N. H. W., Ahmad F., Khong H. Y., Zulkifli R. M. Comparative biological activities of extracts from three Malaysian *Beilschmiedia* species. *Marmara Pharm. J.* 2016; 20 (3): 224-231.

(13) Salleh W. M. N. H. W., Ahmad F. Preliminary investigations of antioxidant, antityrosinase, acetylcholinesterase and anti-inflammatory activities of *Actinodaphne* species. *Marmara Pharm. J.* 2016; 20 (2): 137-143.

(14) Salleh W. M. N. H. W., Hashim N. A., Ahmad F., Khong H. Y. Anticholinesterase and antityrosinase activities of ten *Piper* species from Malaysia. *Adv. Pharm. Bull.* 2014; 4: 527-531.

(15) Tomaino A., Cimino F., Zimbalatti V., Venuti V., Sulfaro V., De Pasquale A., Saija A. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food Chem.* 2005; 89 (5): 549-554.

(16) Braca A., Fico G., Morelli I., Simone F. D., Tome F., Tommasi N. D. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *J. Ethnopharmacol.* 2003; 86 (1): 63-67.

(17) Ismail I., Marjan Z. M., Foong C. W. Total antioxidant activity and phenolic content in selected vegetables. *Food Chem.* 2004; 87 (4): 581-586.

(18) Robards K., Prenzler P. D., Tucker G., Swatsitang P., Glover W. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.* 1999; 66: 401-436.

(19) Harborne J. B., Mendez J. Flavonoids of *Beilschmiedia miersii*. *Phytochemistry* 1969; 8: 763-764.

(20) Lenta B. N., Tantangmo F., Devkota K. P., Wansi J. D., Chouna J. R., Soh R. C., Neumann B., Stammler H. G., Tsamo E., Sewald N. Bioactive constituents of the stem bark of *Beilschmiedia zenkeri. J. Nat. Prod.* 2009; 72:2130-2134.

(21) Amensour M., Bouhdid S., Fernandez-Lopez J., Idaomar M., Senhaji N. S., Abrini J. Antibacterial activity of extracts of *Myrtus communis* against food-borne pathogenic and spoilage bacteria. *Int. J. Food Prop.* 2010; 13 (6): 1215-1224.

(22) Williams R. B., Martin S. M., Hu J. F., Norman V. L., Goering M. G., Loss S., Oneil-Johnson M., Eldridge G. R., Starks C. M. Cytotoxic and antibacterial Beilschmiedic acids from a Gabonese Species of *Beilschmiedia*. *J. Nat. Prod.* 2012; 75: 1319-1325.

(23) Talontsi F. M., Lamshoft M., Bauer J. O., Razakarivony A. A., Andriamihaja B., Strohmann C., Spiteller M. Antibacterial and antiplasmodial constituents of *Beilschmiedia cryptocaryoides*. *J. Nat. Prod.* 2013: 76: 97-102.

(24) Momtaz S., Mapunya B. M., Houghton P. J., Edgerly C., Hussein A., Naidoo S., Lall N. Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening. *J. Ethnopharmacol.* 2008; 119: 507-512.

(25) Mehta J. L., Rasouli N., Sinha A. K., Molavi B. Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction. *Int. J. Biochem. Cell Biol.* 2006; 38: 794–803.

(26) Handoussa H., Hanafi R., Eddiasty I., El-Gendy M., El-Khatib A., Linscheid M., Mahran L., Ayoub N. Anti-inflammatory and cytotoxic activities of dietary phenolics isolated from *Corchorus olitorius* and *Vitis vinifera*. *J. Funct. Foods* 2013; 5: 1204-1216.

(27) Martinez A., Castro A. Novel cholinesterase inhibitors as future effective drugs for the treatment of Alzheimer's disease. *Expert. Opin. Invest. Drugs* 2006; 15: 1-12.

(28) Hirasawa Y., Mitsui C., Uchiyama N., Hakamatsuka T., Morita H. Hupercumines A and B, Lycopodium alkaloids from *Huperzia cunninghamioides*, inhibiting acetylcholinesterase. *Org. Lett.* 2018; 20(5):1384-1387.

(29) Ahmad H., Ahmad S., Shah S. A. A., Latif A., Ali M., Khan F. A., Tahir M. N., Shaheen F., Wadood A., Ahmad M. Antioxidant and anticholinesterase potential of diterpenoid alkaloids from *Aconitum heterophyllum*. *Bioorg. Med. Chem.* 2017; 25 (13): 3368-3376.

(30) Castillo-Ordóñez W., Tamarozzi E. R., da Silva G. M., Aristizabal-Pachón A. F., Sakamoto-Hojo E. T., Takahashi C. S., Giuliatti S. Exploration of the acetylcholinesterase inhibitory activity of some alkaloids from Amaryllidaceae family by molecular docking in silico. *Neurochem Res.* 2017; 42(10): 2826-2830.

غامبل في المختبر الدوائية التقييم من الأوراق ومستخلصات لحاء الجذعية من Beilschmiedia penangiana

محمد سيحرازال احمد[1]، سربية صبية سيد عبد العزيز[2]، فارديه احمد[1]، نورماملة عبدالله[1]، صالح وان حكيمي نزول محمد وان[1]

[1] قسم الكيمياء، كلية العلوم، والرياضيات، أمريكانا جامعة السلطان إدريس السباحة، (35900) تانجونغ ماليم، بيراك، ماليزيا.

[2] قسم الكيمياء، كلية العلوم، طلبه جامعة ماليزيا الجامعة التكنولوجية الماليزية، (81310) جوهور باهرو، جوهور، ماليزيا.

ملخص

تم فحص الأوراق والجذعية اللحاء مقتطفات من بينانجيانا باء لتقييم أنشطتها المضادة للأكسدة، ومضادات الميكروبات، التيروزينات، lipoxygenase، واستيلكولينستراز .وقد تم اختبار تقييم الأنشطة المضادة للأكسدة ضد دبف، أبتس، وفحوصات مجموع محتوى الفينولية .(TPC)، تم التحقيق بنشاط مضادات الميكروبات بواسطة الأسلوب ميكروديلوشن لتحديد هيئة التصنيع العسكري، وأم بي سي و MFC، تم تحليل الأنشطة المثبطة التيروزينات و lipoxygenase استخدام فطر التيروزينات والأنزيمات ليبوكسيجيناسي، على التوالي. بالإضافة إلى ذلك، تم اختبار النشاط أستيلكولينستراز باستخدام إنزيم الكولينستراز. وعند استخراج الميثانول من اللحاء الجذعية أظهر أعلى محتوى الفينولية (176.8) مغ لعبة/ز (دبف الراديكالية الحرة المسح 95.5 IC50) ميكروغرام/مل، (أبتس 124.8 IC50) ميكروغرام/مل، (والنشاط المثبطة) lipoxygenase الأول 46.3 :في المائة. وأظهرت مقتطفات من معتدلة إلى ضعف النشاط مع هيئة التصنيع العسكري/ أم بي سي MFC/ القيم في النطاق من 1000–62.5 ميكروغرام/مل .خلات الإيثيل. أما استخراج اللحاء الجذعية فكشفت عن تثبيط نسبة أعلى في النشاط التيروزينات (الأول 83.2: في المائة)، بينما النشاط أستيلكولينستراز أظهر من الميثانول استخراج أوراق مع تثبيط نسبة 69.4 في المائة وكشفت النتائج أن تستخرج بينانجيانا باء–يمتلك الأنشطة الهامة في مختلف الدراسات البيولوجية .سوف تركز المزيد من التحقيقات في العزلة من نواتج الأيض الثانوية المسؤولة بيواكتيفيتيس.

الكلمات الدالة: بيلشميديا، لاوراسيي، ومضادات الأكسدة، ومضادات الميكروبات، أنتيتيروسيناسي؛ lipoxygenase، أستيلكولينستراز.

تاريخ استلام البحث ٢٠١٨/١/٢٧ وتاريخ قبوله للنشر ٢٠١٨/٨/٨.