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ORIGINAL RESEARCH

Comparative study of biological activities of extracts from three Malaysian *Beilschmiedia* species

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ABSTRACT

This study was carried out to investigate the bioactivities of extracts from three *Beilschmiedia* species, which are *Beilschmiedia glabra* Kosterm, *B. madang* Blume, and *B. pulverulenta* Kosterm. The bioactivity studies were determined on their antioxidant, antimicrobial, antityrosinase, anti-inflammatory, and acetylcholinesterase activities. Evaluation of antioxidant activities were tested against DPPH and phenolic content (TPC) assays. The antimicrobial activity were investigated by microdilution method for determination of MIC, MBC and MFC. Antityrosinase and anti-inflammatory inhibition activity was analysed using mushroom tyrosinase and lipoxygenase enzyme, respectively, while acetylcholinesterase activity against acetylcholinesterase (AChE) enzyme. The MeOH stem bark extract of *B. madang* shows the highest activity on DPPH (IC_{50} of 63.2 µg/mL) and TPC (163.4 mg GA/g) assay. All extracts showed from moderate to weak activity with MIC/MBC/MFC values in the ranged of 125-1000 µg/mL. The EtOAc extract of the leaves of *B. glabra* revealed the highest percentage inhibition on tyrosinase activity (I: 82.5%), while for anti-inflammatory activity showed by the MeOH leaves extract of *B. pulverulenta* (I: 66.6%). The MeOH extract of stem bark of *B. madang* demonstrated the highest activity on acetylcholinestrase activity with percentage inhibition of 62.8%. The results demonstrated that the *Beilschmiedia* species possess significant activities on various biological studies.

Keywords: *Beilschmiedia*, antioxidant, antimicrobial, antityrosinase, anti-inflammatory, acetylcholinesterase, Lauraceae

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INTRODUCTION

Beilschmiedia is a pantropical genus of Lauraceae family with more than 250 species. These plants are native to tropical Asia, Africa, Australia, New Zealand, Central America, Caribbean and South America (1). The genus comprises trees and rarely shrubs and is usually distinguished from the other genera of Lauraceae by the following characteristics: paniculate or racemose inflorescences that are not strictly cymose at the terminal division, bisexual and trimerous flowers with six equal to subequal tepals, six to nine fertile stamens representing the outer two or three whorls, two-celled anthers and fruits lacking cupules (2). In Africa, several *Beilschmiedia* species have been used as remedies in folkloric medicine. *B. manii* is used for the treatment of dysentery and headache, and also as an appetite stimulant (3). In Cameroon, *B. anacardiodes* seeds are used as spice while the stem bark are used as to treat uterine tumors, rubella, female genital infections, and rheumatisms (4). Besides, the fruits of *B. gabonensis* and *B. zenkeri* are used as appetite stimulants

and also as spices. *B. lancilimba* is used in the same region to cure skin bacterial infections (5). In Peninsular Malaysia, a decoction of bark of *B. pahangensis* is used as an imbibe after childbirth and to ease stomachache besides treatment of diarrhea. In addition, the leaves of *B. tonkinensis* are used by the Indonesians and Malays as bandages for broken bones (6). In Madagascar, *B. cryptocaryoides* the fruit, bark, and leaf are utilized by the locals for treatment of contagious disease and malaria (7). Reports on several bioactivities of *Beilschmiedia* species have been published. Earlier reports on the extracts of *B. obscura*, *B. cinnamomea*, *B. tovarensis*, and *B. cinnamomea* have shown antibacterial and antifungal properties (8-10). The extract of *B. erythrophloia* was found as radical scavenger (11). *B. erythrophloia* and *B. tsangii* also displayed potent inhibition on anti-inflammatory activity (12). Other species, such as *B. erythrophloia* and *B. acuta*, were also found as cytotoxic agents, hence potential to be developed for drugs of anti-cancer (13). Based on these findings, there is a need to investigate other *Beilschmiedia* species from other origins to further develop the standardized extracts for specific medicinal purposes in the future.

We herein present the first report on the systematic studies of biological activities of three *Beilschmiedia* species originated from Malaysia. This study focused on the analysis of antioxidant, antimicrobial, antityrosinase, anti-inflammatory and acetylcholinesterase activities of the leaves and stem bark extracts of *B. glabra*, *B. madang*, and *B. pulverulenta*.

MATERIAL AND METHODS

Plant materials and extraction method

B. glabra (Voucher no. SK2570/14) (leaves: 1 kg, stem bark: 2 kg) was collected from Kluang, Johor in October 2014, while *B. madang* (Voucher no. SK1984/12) (leaves: 1 kg, stem bark: 2 kg) was collected from Bangi, Selangor, in September 2012. The species were identified by Dr. Shamsul Khamis (UPM) and the voucher specimens were deposited at the Herbarium of IBS, UPM. *B. pulverulenta* (Voucher no. UiTMKS4014) (leaves: 0.5 kg, stem bark: 0.3 kg) was collected from Kota Samarahan, Sarawak and identified by Mohizar Mohamad (UiTMKS). The voucher specimen was deposited at NPRDC, UiTM Sarawak. The dried and powdered leaves and stem bark of the above mentioned *Beilschmiedia* species were extracted firstly with *n*-hexane (Hex), followed by ethyl acetate (EtOAc) and methanol (MeOH). 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 all plant extracts obtained are shown in Table 1.

Table 1. Percentage yield and antioxidant activities of extracts of *Beilschmiedia* species

Species/Part	Extracts	Yield	DPPH IC_{50} (μg/mL)	TPC (mg GA/g)
B. glabra Leaves	Hex	5.0 g, 0.50%	221.6	51.5 ± 0.2
	EtOAc	10.8 g, 1.08%	209.7	40.1 ± 0.1
	MeOH	18.1 g, 1.81%	130.1	53.4 ± 0.2
B. glabra Stem bark	Hex	15.4 g, 0.77%	275.1	47.7 ± 0.2
	EtOAc	18.9 g, 0.95%	98.0	145.8 ± 0.2
	MeOH	25.3 g, 1.27%	134.9	54.4 ± 0.3
B. madang Leaves	Hex	13.5 g, 0.68%	192.2	49.2 ± 0.3
	EtOAc	16.3 g, 0.82%	152.4	41.5 ± 0.4
	MeOH	20.9 g, 1.05%	104.5	114.5 ± 0.4
B. madang Stem bark	Hex	14.5 g, 0.73%	101.5	157.6 ± 0.4
	EtOAc	26.2 g, 1.31%	110.2	147.6 ± 0.5
	MeOH	28.1 g, 1.41%	63.2	163.4 ± 0.2
B. pulverulenta Leaves	Hex	4.9 g, 0.99%	79.5	132.1 ± 0.3
	EtOAc	5.2 g, 1.05%	74.5	148.5 ± 0.3
	MeOH	6.4 g, 1.30%	65.7	162.6 ± 0.4
B. pulverulenta Stem bark	Hex	5.0 g, 1.67%	133.3	80.4 ± 0.2
	EtOAc	8.7 g, 2.90%	128.7	96.2 ± 0.4
	MeOH	7.2 g, 2.40%	71.6	161.5 ± 0.4
BHT			18.5	-

TPC - Total phenolic content; 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). Antityrosinase: Mushroom tyrosinase enzyme (EC1.14.18.1), kojic acid and L-dopa were purchased from Sigma-Aldrich (Germany). Anti-inflammatory: 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

Total phenolic content (TPC) assay

TPC of the extracts was measured using gallic acid equivalent with minor modifications (14). 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 3 min, 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. Tests were carried out in triplicate and the gallic acid equivalent value was reported as mean ± SD of triplicate.

DPPH free radical scavenging assay

The free radical scavenging activity was measured by the DPPH method with minor modifications (15). 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 percent 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 were carried out in triplicate and IC_{50} values were reported as means ± SD of 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 were 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 (14). 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 well 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.

Antityrosinase activity

Tyrosinase inhibition assay was carried out following the standard method (16-17) with slight modifications. 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.

Anti-inflammatory 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 (18-19). 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. A statistical package (SPSS version 11.0) was used for the data analysis.

RESULTS AND DISCUSSION

The DPPH radical scavenging and total phenolic content were chosen to gain understanding of the true antioxidant potential of the extracts. The antioxidant activity of the

three *Beilschmiedia* species is summarised in Table 1. In the DPPH assay, the highest activity was found from the MeOH stem bark extract of *B. madang* with IC_{50} value of 63.2 µg/mL. In addition, the hex, EtOAc and MeOH extracts of *B. pulverulenta* were also found to be good radical scavengers with IC_{50} values of 65.7, 74.5 and 79.5 µg/mL, respectively. However, these values were lower than the positive control, BHT which showed IC_{50} value of 18.5 µg/mL. The MeOH stem bark extract of *B. madang* was found to have the highest TPC (163.4 mg GA/g), followed by the MeOH leaves extract of *B. pulverulenta* (162.6 mg GA/ g) and their stem bark extract (161.5 mg GA/g). Phenolic compounds are known as plants antioxidant agents due to their capability as reducing agents, hydrogen donors and singlet oxygen scavengers (20-21). Phenolic compounds such as flavonoids are noted as antioxidant agents. The plant extracts also contained some flavonoids as reported previously from *B. zenkeri*, *B. tovarensis* and *B. miersii* (22-23). The phenolic groups of flavonoids are hydrogen donator to scavenge free radicals (24).

Table 2 shows the MIC, MBC and MFC of plant extracts against nine microorganisms. Most of the 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*). For antifungal activity, the best activity was showed by the hex and MeOH leaves extract of *B. glabra* with MIC value of 125 µg/mL. Antimicrobial activity of the extracts against the Gram-negative bacteria were found weak activity compare to Gram-positive bacteria, due to thick outer lipid bilayer with embedded proteins and attached polysaccharides (25). Several reported compounds from the genus *Beilschmiedia* were found to have antimicrobial activities. Compounds with antifungal and antibacterial activities have been isolated from other species of *Beilschmiedia.* They are beilschmiedic acid A, beilschmiedic acid K and beilschmiedic acid L, which were isolated from the bark extracts of *B. gabonese*. These compounds were reported to be active against clinical isolate of methicillin-resistant *Staphylococcus aureus* (26). Cryptobeilic acid A and cryptobeilic acid B isolated from *B. cryptocaryoides* have showed antibacterial activity against *Escherichia coli*, *Acinetobacter calcoaceticus* and *Pseudonamas stutzeri* (7). Pipyahyine, an amide isolated from *B. zenkeri* showed moderate activity against *Bacillus subtilis* and *Pseudomonas agarici*, while a flavonoid, beilschmieflavonoid B showed weak activity against *Streptococcus minor* (23). 2-Hydroxy-9-methoxyaporphine, a new alkaloid isolated from *B. alloiophylla* showed significant antifungal activity against *Candida albicans* (27).

Table 2. Antimicrobial activity of extracts of *Beilschmiedia* species

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
B. glabra leaves	Hex	250	250	500	1000	125	250	250	500	250	250	250	500	500	500	250	500	250	250
	EtOAc	125	250	500	1000	125	250	250	500	250	500	250	500	500	500	125	250	250	250
	MeOH	125	250	125	250	125	250	125	250	250	500	125	250	250	250	125	250	250	250
B. glabra stem bark	Hex	500	500	1000	1000	125	250	500	500	500	500	500	1000	125	125	250	500	125	125
	EtOAc	500	500	500	1000	125	250	250	500	250	500	500	1000	250	250	250	500	250	250
	MeOH	250	500	500	500	125	250	500	500	250	500	250	500	125	125	125	125	125	125
B. madang leaves	Hex	500	1000	250	500	500	1000	1000	1000	1000	1000	1000	1000	500	1000	1000	1000	1000	1000
	EtOAc	500	1000	500	500	500	1000	1000	1000	500	1000	1000	1000	500	1000	500	1000	500	1000
	MeOH	125	500	125	250	125	250	1000	1000	500	1000	1000	1000	500	1000	500	1000	500	1000
B. madang stem bark	Hex	500	1000	125	250	500	1000	500	1000	500	1000	250	500	250	500	1000	>1000	1000	1000
	EtOAc	250	500	250	500	250	500	500	1000	500	1000	250	500	500	1000	1000	>1000	1000	1000
	MeOH	250	500	125	250	500	1000	500	1000	250	500	500	1000	250	500	1000	>1000	1000	1000
B. pulveru-lenta leaves	Hex	250	500	250	500	250	500	250	500	250	500	500	1000	250	500	250	500	500	500
	EtOAc	250	500	250	500	500	1000	500	1000	500	1000	500	1000	125	250	500	1000	250	500
	MeOH	125	250	125	250	125	250	500	250	250	500	250	500	125	250	500	1000	250	500
B. pulveru-lenta stem bark	Hex	500	1000	500	1000	500	1000	500	1000	250	500	500	1000	125	250	1000	1000	250	500
	EtOAc	500	1000	500	1000	500	1000	500	1000	250	500	500	1000	125	250	1000	1000	250	500
	MeOH	500	1000	500	1000	500	1000	500	1000	250	500	500	1000	125	250	1000	1000	250	500
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 summarizes the results of inhibition percentage of antityrosinase, anti-inflammatory and acetylcholinesterase inhibition activity at the concentration of 1 mg/mL. Antityrosinase inhibition activity was analysed using mushroom tyrosinase. The EtOAc and MeOH extract of *B. glabra* displayed the highest tyrosinase inhibition activity which gave 82.5% and 82.3% inhibition, respectively. These results are slightly lower than that of kojic acid which revealed inhibition of 97.1%. Gallic acid, (-)-epicatechin and procyanidin B2 are examples of phenolic acids and flavonoids that are known as tyrosinase inhibitors. The formation of hydrogen bonding of the available hydroxyl groups of these compounds with the active site of the enzyme is suggested as the key factor for tyrosinase inhibition (28-30).

Table 3. Antityrosinase, anti-inflammatory, and acetylcholinesterase inhibitory activities of extracts of *Beilschmiedia* species

Species	Extracts	Antityrosinase (I%)	Anti-inflammatory (I%)	Acetylcholinesterase (I%)
B. glabra Leaves	Hex	73.0 ± 0.3	58.0 ± 0.2	29.5 ± 0.5
	EtOAc	82.5 ± 0.2	56.2 ± 0.3	39.4 ± 0.4
	MeOH	82.3 ± 0.2	61.1 ± 0.2	47.8 ± 0.3
B. glabra Stem bark	Hex	58.2 ± 0.1	47.3 ± 1.5	39.2 ± 0.5
	EtOAc	68.5 ± 0.2	48.4 ± 0.3	45.6 ± 0.3
	MeOH	78.5 ± 0.1	63.1 ± 1.2	57.5 ± 0.4
B. madang Leaves	Hex	59.8 ± 0.4	38.8 ± 0.2	35.5 ± 0.4
	EtOAc	50.6 ± 0.3	38.9 ± 0.2	43.2 ± 0.3
	MeOH	60.5 ± 0.2	43.4 ± 0.2	54.2 ± 0.4
B. madang Stem bark	Hex	45.2 ± 0.2	32.0 ± 1.2	44.2 ± 0.3
	EtOAc	52.6 ± 0.2	45.2 ± 1.5	45.5 ± 0.2
	MeOH	58.2 ± 0.2	47.8 ± 1.3	62.8 ± 0.2
B. pulverulenta Leaves	Hex	40.6 ± 0.2	54.1 ± 0.8	30.4 ± 0.4
	EtOAc	48.6 ± 0.3	61.0 ± 0.6	32.5 ± 0.3
	MeOH	60.1 ± 0.3	66.6 ± 0.5	43.2 ± 0.5
B. pulverulenta Stem bark	Hex	41.8 ± 0.2	35.0 ± 1.1	40.5 ± 0.5
	EtOAc	62.3 ± 0.2	42.6 ± 0.2	50.4 ± 0.4
	MeOH	65.2 ± 0.2	42.4 ± 0.2	57.8 ± 0.3
Kojic acid		97.1 ± 0.1	-	-
Quercetin		-	89.1 ± 0.2	-
Galantamine		-	-	95.9 ± 0.2

Data represent as mean ± SD of three independent experiments; I% - percentage inhibition

Anti-inflammatory inhibition activity was analysed using lipoxygenase enzyme. In anti-inflammatory activity, a standard lipoxygenase inhibitory chemical, quercetin, inhibited lipoxygenase activity by 89.1% at 1 mg/mL. The MeOH and EtOAc extracts of *B. pulverulenta* exhibited moderate lipoxygenase inhibitory effect of 66.6% and 61.0%, respectively, followed by the hex and EtOAc extracts of *B. glabra* with 58.0% and 56.2% of inhibitory activity, respectively. However, the extracts of *B. madang* showed weak inhibition ranging from 13.4-18.9%. LOX catalyzes dioxygenation of polyunsaturated fatty acids to yield *cis*, *trans*-conjugated diene hydroperoxides. It is worth mentioning that, the extracts of *B. pulverulenta* had the highest TPC and significant radical scavenger, which is consistent with a previous study which found a relationship between the anti-inflammatory activity and the presence of polyphenols (31). Antioxidants are also known to inhibit plant lipoxygenases. Studies have implicated that oxygen free radicals in the process of inflammation and phenolic compounds may block the cascade process of arachidonic acid metabolism by inhibiting lipoxygenase activity and serve as a scavenger of reactive free radicals that are produced during arachidonic acid metabolism (32-33).

The extracts were screened against acetylcholinesterase inhibitory activity using the enzyme of AChE and acetycholine iodide as a substrate. Galantamine was used as standard. In acetylcholinesterase activity, the MeOH stem bark extract of *B. madang* exerted the highest AChE activity with inhibition of 62.8%. 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 (34). Since the strongest synthetic or natural product driven AChE inhibitors are known to contain nitrogen, the activity of the extracts could be due to their alkaloidal contents (35-36). These results were in accordance with the previous phytochemical studies of *Beilschmiediea* species which found to have alkaloids as their major compounds. It has been isolated phytochemically from *B. kunstleri* (37), *B. alloiophylla* (27), *B. oreophila* (38), *B. brevipes* (39), and *B. podagrica* (40).

CONCLUSION

In summary, the present paper reports for the first time a systematic comprehensive study on the antioxidant, antimicrobial, antityrosinase, anti-inflammatory, and acetylcholinesterase activities on extracts of *Beilschmiedia* species. As a consequence, identification of the active principles using bioassay-guided isolation from these species and in-depth studies of their pharmacology safety are warranted to capitulate potential new therapeutic drugs or drug leads.

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Malezya'da yetişen *Beilschmiedia* türlerinden hazırlanan üç ekstrenin biyolojik etkilerinin karşılaştırılması

ÖZ

Bu çalışma *Beilschmiedia glabra*, *B. madang* ve *B. pulverulenta* olmak üzere *Beilschmiedia* türünden üç ekstrenin biyolojik etkinliklerini incelemek için yapılmıştır. Biyolojik etki çalışmaları kapsamında, ekstreler, antioksidan, antimikrobiyal, antitirozinaz, anti-enflamatuvar ve asetilkolinesteraz etki potansiyelleri açısından değerlendirilmiştir. Antioksidan etki değerlendirilmesi DPPH ve fenolik içeriği (TCK) deneyleri ile yapılmıştır. Antimikrobiyal etki MIC, MBC ve MFC belirlenmesi için mikrodilüsyon yöntemiyle incelendi. Antitirozinaz ve anti-inflamatuar aktivitesi inhibisyonu sırasıyla mantar tirozinaz ve lipoksijenaz enzimi kullanarak asetilkolinesteraz aktivitesi asetilkolinesteraz (AChE) enzimine karşı analiz edilmiştir. *B. madang* MeOH gövde kabuğu ekstresi DPPH (63.2 mg / ml IC50) ve TPC (163.4 mg, GA / g) deneyinde en yüksek aktivite göstermiştir. Tüm ekstreler ortadan zayıfa doğru ve 125-1000 mg / mL MIC / MBC / MFC değerleri arasında etkinlik gösterdi. *B. glabra* yapraklarının EtOAc ekstresi tirozinaz aktivitesi üzerinde en yüksek inhibitor aktivite (I: %82.5) göstermişken *B. pulverulenta* yapraklarının MeOH ekstresi en yüksek antiinflamatuar aktiviteyi göstermiştir. (I:% 66.6). *B. madang* kök kabuğu MeOH ekstresi %62.8 inhibisyonu yüzdesi ile asetilkolinesteraz aktivitesi üzerine en yüksek etkinliği göstermiştir. Sonuçlar *Beilschmiedia* türlerinin çeşitli biyolojik çalışmalar üzerinde önemli aktiviteleri olduğunu ortaya koymuştur.

Anahtar kelimeler: *Beilschmiedia*, antioksidan, antimikrobiyal, antitirozinaz, anti-enflamatuar, asetilkolinesteraz

REFERENCES

1. Nishida WS. Revision of *Beilschmiedia* (Lauraceae) in the Neotropics. Ann Missouri Bot Gard 1999; 86: 657-701.
2. Nishida WS. Taxonomic Revision of *Beilschmiedia* (Lauraceae) in Borneo. Blumea 2008; 53: 345-83.
3. Iwu MM. Handbook of African Medicinal Plants. CRC Press, Boca Raton, FL. 1993.
4. Tchouala JM. Inventaire Ethnobotanique des plantes medicinales du Noun (Ouest-Cameroun) utilisees pour soigner les infections fongiques et bacteriennes, Memoire de Maitrise de Biologie Vegetale. Universite de Dschang. 2001.
5. Nkeng-Efouet PA. Phytochemicals from *Beilschmiedia anacardioides* and their biological significance, phytochemicals-A global perspective of their role in nutrition and health. Editor: Venketeshwer R., 2012.
6. Wiart C. Medicinal Plants of Asia and the Pacific. Taylor & Francis, Boca Raton, London, New York. 2006.
7. Talontsi FM, Lamshoft M, Bauer JO, Razakarivony AA, Andriamihaja B, Strohmann C, Spiteller M. Antibacterial and antiplasmodial constituents of *Beilschmiedia cryptocaryoides*. J Nat Prod 2013; 76: 97-102.
8. Suarez LEC, Vargas OEB. New chalcones from *Beilschmiedia tovarensis*. Rev Colomb Quim 2005; 34: 35-41.
9. Fankam AG, Kuete V, Voukeng IK, Kuiate JR, Pages JM. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. BMC Complement Altern Med 2011; 11: 104-14.

10. Fankam AG, Kuiate JR, 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: 241-50.
11. Lin CT, Chu FH, Tseng YH, Tsai JB, Chang ST, Wang SY. Bioactivity investigation of Lauraceae trees grown in Taiwan. Pharm Biol 2007; 45: 638-44.
12. Huang YT, Chang HS, Wang GJ, Cheng MJ, Chen CH, Yang YJ, Chen IS. Anti-inflammatory endiandric acid analogues from the roots of *Beilschmiedia tsangii*. J Nat Prod 2011; 74: 1875-80.
13. Kuete V, Tankeo SB, Saeed ME, Wiench B, Tane P, Efferth T. Cytotoxicity and modes of action of five Cameroonian medicinal plants against multi-factorial drug resistance of tumor cells. J Ethnopharmacol 2014; 153: 207-19.
14. Salleh WMNHW, Ahmad F, Khong HY. Chemical compositions and biological activities of the essential oils of *Beilschmiedia madang* Blume (Lauraceae). Arch Pharm Res 2015; 38: 485-93.
15. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthine on autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992; 40: 945-8.
16. Masuda T, Yamashita D, Takeda Y, Yonemori S. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. Biosci Biotech Biochem 2005; 69: 197-201.
17. Salleh WMNHW, Hashim NA, Ahmad F, Khong HY. Anticholinesterase and antityrosinase activities of ten *Piper* species from Malaysia. Adv Pharm Bull 2014; 4: 527-31.
18. Ellman GL, Courtney KD, Andres V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88-95.
19. Orhan I, Aslan S, Kartal M, Sener B, Baser KHC. Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes. Food Chem 2008; 108: 663-8.
20. Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W. Phenolic compounds and their role in oxidative processes in fruits. Food Chem 1999; 66: 401-36.
21. Hakkim FL, Shankar CG, Girija S. Chemical composition and antioxidant property of holy basil (*Ocimum sanctum* L.) leaves, stems and inflorescence and their in vitro callus cultures. J Agric Food Chem 2007; 55: 9109-17.
22. Harborne JB, Mendez J. Flavonoids of *Beilschmiedia miersii*. Phytochemistry 1969; 8: 763-4.
23. Lenta BN, Tantangmo F, Devkota KP, Wansi JD, Chouna JR, Soh RC, Neumann B, Stammler HG, Tsamo E, Sewald N. Bioactive constituents of the stem bark of *Beilschmiedia zenkeri*. J Nat Prod 2009; 72: 2130-4.
24. Brand-Williams W, Cuvelier ME, Berset C). Use of a free-radical method to evaluate antioxidant activity. LWT Food Sci Tech 1995; 28: 25-30.
25. McKee T, McKee JR. Biochemistry The Molecular Basis of Life, 3rd Ed., McGraw-Hill, USA. 2003.
26. Williams RB, Martin SM, Hu JF, Norman VL, Goering MG, Loss S, Oneil-Johnson M, Eldridge GR, Starks CM. Cytotoxic and antibacterial Beilschmiedic acids from a Gabonese Species of *Beilschmiedia*. J Nat Prod 2012; 75: 1319-25.
27. Mollataghi A, Hadi AH, Shiau-Chuen C. (+)-Kunstleramide, a new antioxidant and cytotoxic dienamide from the bark of *Beilschmiedia kunstleri* Gamble. Molecules 2012; 17: 4197-4208.
28. Kubo I, Chen QX, Nihei KI. Molecular design of antibrowning agents: antioxidative tyrosinase inhibitor. Food Chem 2003; 81: 241-7.
29. Momtaz S, Mapunya BM, Houghton PJ, 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: 507-12.
30. Alam N, Yoon KN, Lee KR, Kim HY, Shin PG, Cheong JC, Yoo YB, Shim MJ, Lee MW, Lee TS. Assessment of antioxidant and phenolic compound concentrations as well as xanthine oxidase and tyrosinase inhibitory properties of different extracts of *Pleurotus citrinopileatus* fruiting bodies. Mycobiol 2011; 39: 12-19.
31. Handoussa H, Hanafi R, Eddiasty I, El-Gendy M, El-Khatib A, Linscheid M, Mahran, Ayoub N. Anti-inflammatory and cytotoxic activities of dietary phenolics isolated from *Corchorus olitorius* and *Vitis vinifera*. J Funct Foods 2013; 5: 1204-16.
32. Lin JK, Tsai SH, Lin-Shiau SY. Anti-inflammatory and antitumor effects of flavonoids and flavanoids. Drugs Fut 2001; 26: 145-57.
33. Trouillas P, Calliste CA, Allais DP, Simon A, Marfak A, Delage C, Duroux JL. Antioxidant, anti-inflammatory and anti-proliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. Food Chem 2003; 80: 399-407.
34. Zarotsky V, Sramek JJ, Culter NR. Galanthamine hydrobromide: an agent for Alzheimer's disease. Am J Health-System Pharmacist 2003; 60: 446-52.
35. 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.
36. Mukherjee PK, Kumar V, Mal M, Houghton PJ. Acetylcholinesterase inhibitors from plants. Phytomedicine 2007; 14: 289-300.
37. Mollataghi A, Coudiere E, Hadi AHA, Mukhtar MR, Awang K, Litaudon M, Ata A. Anti-acetylcholinesterase, anti-α-glucosidase, antileishmanial and antifungal activities of chemical constituents of *Beilschmiedia* Species. Fitoterapia 2012; 83: 298-302.
38. Tillequin F, Koch M, Pusset J, Chauviere G. Two new morphinane alkaloids from *Beilschmiedia oreophila* Schlechter (Lauraceae). Heterocycles 1985; 6: 1357-61.
39. Pudjiastuti P, Mukhtar MR, Hadi AH, Saidi N, Morita H, Litaudon M, Awang K. (6,7-Dimethoxy-4-methylisoquinolinyl)-(4′-methoxyphenyl)-methanone, a new benzylisoquinoline alkaloid from *Beilschmiedia brevipes*. Molecules 2010; 15: 2339-46.
40. Johns SR, Lamberton JA, Sioumis AA, Tweeddale HJ. New Aporphine alkaloids from *Beilschmiedia podagrica*. Aust J Chem 1969; 22: 1277-81.