BRJ BASIC RESEARCH JOURNALS

Basic Research Journal of Microbiology ISSN 2354-4082 Vol. 6(1) pp. 16-22 November 2019
Available online http//www.basicresearchjournals.org

Full Length Research Paper

Phytochemical studies and their bioactivities from *Beilschmiedia Palembanica (Miq.)* Kosterm

Abbas Mollataghi[1, 2,*]

[1]Department of chemistry, Faculty of Science, Qom University of Technology, Qom, Iran.
[2]Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia.

*Author's eMail: a_mollataghi2@yahoo.com; Tel.: +98-912-6528961

Acceptance 11 November, 2019

ABSTRACT

(+)-kunstlerone (1) as a neolignan, together with nine known alkaloids: (+)-norboldine (2), isocaryachine (3), pseudocodamine (4), milonine (5), sebiferine (6) and (-)-salutarine (7), noratherosperminine (8), 10-O-methyl hernovine (9), atherosperminine (10), and the amide was cinnamide (11), were isolated from the leaves of *Beilschmiedia Palembanica (Miq.) Kosterm.* The structures were established through various spectroscopic methods notably 1D- and 2D-NMR, UV, IR and LCMS-IT-TOF. (+)- Kunstlerone (1) showed a strong antioxidant activity, with an SC_{50} of 20.0 µg/mL Additionally, the effect of compounds 1, 2 and 3 were evaluated on A549, PC-3, A375, HT-29 and WRL-68 cell lines. Kunstlerone 1 showed moderate cytotoxicity against various cancer cell lines such as A549, PC-3, A375, HT-29 and WRL-68, respectively with EC_{50} values of 28.02, 26.78, 33.78, 33.65 and 16.46 µg/mL. The crude methanol extract showed antigrowth activity against *S. pyogenes* II and *B. cereus*, with MICs of 256 µg/mL. The compounds kunstlerone (1), isocaryachine (3) and noratherosperminine (8) showed complete inhibition against *P. shigelloides*, with MIC ≤ 60 µg/mL compare to ampicillin, as a positive control, which showed antigrowth activity against *P. shigelloides* at MIC 10 µg/mL.

Keywords: *Beilschmiedia Palembanica*, alkaloid, neolignan, lauraceae, antioxidant, anticancer

INTRODUCTION

The Lauraceae family normally, with 40 genera and over 2,000 species, occurs throughout Southeast Asia and tropical America (Soepadmo, 1999; Corner, 1988). In Malaysia, its contribution is about 213 species, from 16 genera (Corner, 1988). *Beilschmiedia* species are known to produce many types of phytochemicals (Lenta et al., 2009; Guinaudeau et al.,1979; Chouna et al.,2009; Harborne and Méndez, 1969; Yang et al., 2009; Yang et al., 2008; Chen et al., 2007; Setzer and Haber, 2007; Kitagawa et al., 1993), with varied biological activities such as *O,O*-dimethylcoclaurine isolated from Malaysian *B. brevipes,* which exhibited significant cytotoxicity against P-388 murine leukemia cells with an IC_{50} value of 6.5 g/mL (Pudjiastuti et al., 2010). Besides the alkaloids, epoxyfuranoid lignans were also reported in the leaves of *B. tsangii* (Chen et al., 2007). Neolignans, whose precursors are the di- and trioxygenated cinnamic acids, have never been reported so far in the species of *Beilschmiedia;* however they were detected in parts of other species of Lauraceae such as in the fruits of *Aniba riparia* and in the leaves of *Ocotea catharinensis* (Rossi et al., 1997; Barbosa et al., 1987; Funasaki et al., 2009).

In continuation of our search for new bioactive compounds from Malaysian flora, Pudjiastuti et al. (2010); Rachmatiah et al. (2009) we have performed a phytochemical study on the leaves of a Malaysian

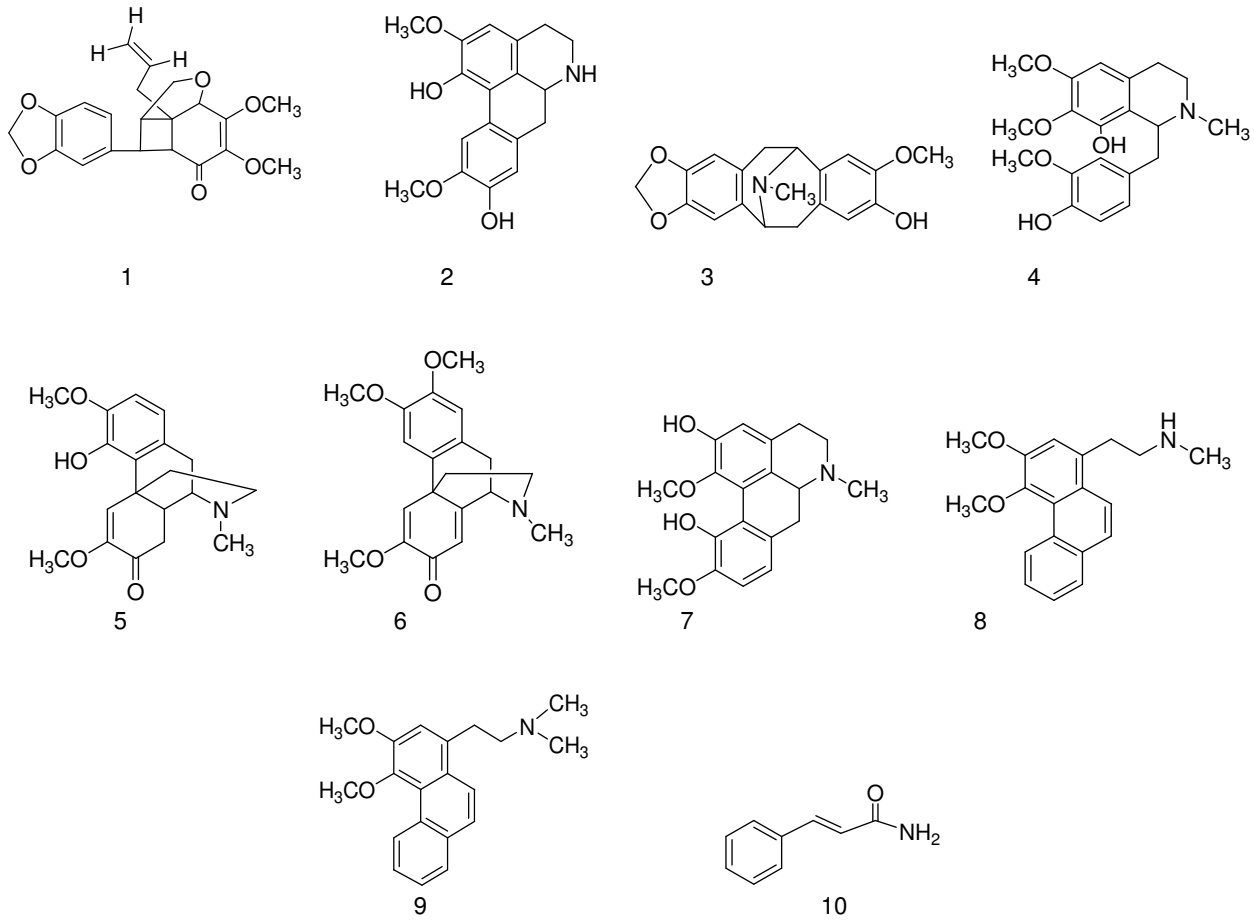

Figure 1. Chemical structures of compounds **1-11**.

Lauraceae, *Beilschmiedia Palembanica (Miq.) Kosterm*, which has led to the isolation of (+)-kunstlerone (1) as a neolignan, together with nine known alkaloids: (+)-norboldine (2), isocaryachine (3), pseudocodamine (4), milonine (5), sebiferine (6) and (-)-salutarine (7), noratherosperminine (8), 10-O-methyl hernovine (9), atherosperminine (10), and the amide was cinnamide (11), were also isolated (Figure 1). This paper describes the structural elucidation and the DPPH radical scavenging activity and cytotoxicity against various cancer cell lines such as A549, PC-3, A375, HT-29 and WRL-68 of (+)-kunstlerone (1), isocaryachine (3) and noratherosperminine (8).

RESULTS AND DISCUSSION

Antioxidant activity

(+)- Kunstlerone (1) was tested for antioxidant activity using a DPPH radical scavenging activity assay. The results have shown that it possesses a potent antioxidant activity, with scavenging capacity (SC_{50} = 20.0µg/mL) comparable to that of ascorbic acid (SC_{50} = 14.0µg/mL, Figure 2). The antioxidant activities of neolignan glycosides from *Verbascum salviifolium* Boiss and neolignan phenols from *Nectandra grandiflora* have been reported previously (Ribeiro et al., 2005). The mechanism of action for the strong scavenging capacity of compound 1 is under investigation.

Antimicrobial activity studies

Antimicrobial activity of the extracts and isolates were investigated using the method of agar dilution. Provisionally, the tested compounds dissolved in either CH_2Cl_2 or MeOH were individually diverse with Muller Hinton (MH) broth to obtain a final volume of 2mL. Two-fold dilution was prepared and the solution was then transferred to the agar solution of MH to yield the final concentrations ranging from 256µg/mL, Twenty seven strains of microorganisms, refined in MH broth at 30°C for 24hrs, were diluted with 0.9% normal saline solution to regulate the cell density of 108 CFU/mL

Microorganisms

The organisms were inoculated against each plate using

Table 1. The organisms were inoculated against each plate using a multipoint inoculators.

Folder	strains of microorganisms
Gram(-) bacteria	*Escherichia coli ATCC 25922* *Klebsiella pneumoniae ATCC 700603* *Serratia marcescens A TCC 8100* *Salmonella typhimurium ATCC 13311* *Salmonella choleraesuis ATCC 10708* *Shewanella putrefaciens ATCC 8671* *Achromobacter xylosoxidans A TCC 2706* *Pseudomonas aeruginosa ATCC 15442* *Pseudomonas stutzeri ATCC 17587*
Gram(+) bacteria	*Staphylococcus aureus ATCC 29213* *Staphylococcus aureus ATCC 25923* *Enterococcus faecalis A TCC 29212* *Enterococcus faecalis ATCC 33186* *Micrococcus lutens A TCC 10240* *Bacillus subtilis ATCC 6633* *Corynebacterium diphtheriae NCTC 10356*
Clinical specimens Gram(-) bacteria	*Shigella dysenteriae* *Salmonella enteritidis type C* *Morganella morganii* *Aeromonas hydrophila* *Citrobacter freundii* *Plesiomonas shigelloides*
Clinical specimens Gram(+) bacteria	*Streptococcus pyogenes II* *Bacillus cereus* *Listeria monocytogenes*
Yeasts	*Saccharomyces cereviseae ATCC 2601* *Candida albicans ATCC 90028*

a multipoint inoculators and further incubated at 30°C for 18-48hrs. Compounds which possessed high efficacy to inhibit bacterial cell growth were analyzed (Table 1). As aspect, these microorganism strains has assume that which strain has ability for interchanging and which sourcing the strains from different culture folders has not any effect against result the media quality results and qualification test methods.

Cytotoxic activity studies

Cell culture

All the cells that applied in this study were obtained from American Type Cell Collection (ATCC) and maintained in a 37°C incubator with 5% CO_2 saturation. A375 human melanoma, HT-29 human colon adenocarcinoma cells and WRL-68 normal hepatic cells were maintained in Dulbecco's modified Eagle's medium (DMEM). Whereas A549 non-small cell lung cancer cells and PC-3 prostate adenocarcinoma cells were maintained in RPMI medium. Both medium were supplemented with 10% fetus calf serum (FCS), 100 units/mL penicillin, and 0.1 mg/mL streptomycin.

Cellular viability

Different cell types from above were used to find out the inhibitory effect of compounds kunstlerone (1), isocaryachine (3), and noratherosperminine (8) on cell growth using the MTT assay. Briefly, cells were seeded at a density of 1×10^5 cells/mL in a 96-well plate and incubated for 24 hours at 37°C, 5% CO_2. Next day, cells were treated with the compounds respectively and incubated for another 24 hours. After 24 hours, MTT solution at 2 mg/mL was added for 1 hour. Absorbance at 570 nm was measured and recorded using Plate Chameleon V microplate reader (Hidex, Turku, Finland). Results were expressed as a percentage of control giving percentage cell viability after 24 hours exposure to test agent. The potency of cell growth inhibition for each test agent was expressed as an EC_{50} value, defined as the concentration that caused a 50% loss of cell growth. Viability was defined as the ratio (expressed as a

Table 2. Strains of microorganisms

Sample	Microorganism	Gram	MIC (µg/mL)
Crude hexane extract	*C. diphtheriae NCTC 10356*	+	256
	C. diphtheriae NCTC 10356	+	256
	A. xylosoxidan ATCC 2706	-	256
	S. aureus ATCC 25923	+	256
Crude dichloromethane extract	*M lutens ATCC 10240*	+	256
	A. hydrophila	-	256
	B. cereus	+	256
	S. putrefaciens ATCC 8671	-	128
	C. diphtheriae NCTC 10356	+	256
Crude methanol extract	*S. pyogenes II*	+	256
	B. cereus	+	256
Ampicillin	*P. shigelloides*	-	10

percentage) of absorbance of treated cells to untreated cells (Braga et al., 2007).

Antimicrobial activity

The crude dichloromethane, hexane and methanol extracts, and the isolates (1, 3 and 8) from leaves of *Beilschmiedia palembanica* were experienced and tested for antimicrobial activity against 15 strains of microorganisms by means of the agar dilution method (Okusa et al., 2007). The domino effect (Table 2) give us an idea about that all the tested extracts entirely inhibit the expansion of the Gram-positive bacterium *C. diphtheriae* NCTC 10356 with MIC 256 µg/mL. This strain was the most sensitive, as it turned out the only one inhibited by the crude hexane and dichloromethane extracts. Other microorganisms such as *A. xylosoxidan* ATCC 2706, *S. aureus* ATCC 25923, *M. lutens* ATCC 10240, *B. cereus* and *A. hydrophila* were wholly inhibited by the crude dichloromethane extract, with MIC 256 µg/mL. In addition, the crude methanol extract displays antigrowth activity against *S. pyogenes* II and *B. cereus*, with MICs of 256 µg/mL.

In the current research, the antimicrobial activity of pure compounds (1,3 and 8) isolated from the dichloromethane and methanol extract was similarly evaluated. It was found (Table 1) that compounds 1, 3 and 8 displayed complete inhibition against *P. shigelloides*, with MIC ≤ 60 µg/mL. In evaluation ampicillin, a positive be in charge, showed antigrowth doings against *P. shigelloides* at MIC 10µg/mL.

Cytotoxic activity

To evaluate the cytotoxic activity, each different compound namely kunstlerone (1), isocaryachine (3) and noratherosperminine (8) were tested with a series of different doses on A549, PC-3, A375, HT-29 and WRL-68, respectively (Figure 3). After 24 hours, cell viability was determined by the MTT assay. Test agents induced cell cytotoxicity in a concentration dependent manner. These dose titration curves allowed determining EC_{50} for the test agents towards different cell lines (Table 3).

From Figure 3, isocaryachine (3) and noratherosperminine (8) showed no cytotoxic effect on various cell lines. On the other hand, Kunstlerone (1) also showed cytotoxic effect on several of the cancer cell lines with different EC_{50} valuesin a concentration dependent manner. These dose titration curves allowed determining EC_{50} for the various compounds towards different cell lines. Kunstlerone (1) demonstrated dose-depended cytotoxic effects with EC_{50} values of 33.78 ± 3.4, 28.02 ± 1.7, 33.65 ± 0.9, 26.78 ± 2.3 and 16.46 ± 0.7 µg/mL; in A375, A549, HT-29, PC-3 and WRL-68, respectively. These results indicate that cell lines differ in their sensitivity to the same test agent, which may be determined by multiple cell type-specific signalling cascades and transcription factor activities.

Cytotoxic screening models make available important preliminary data to select plant extracts or natural compounds with potential anticancer properties. In this study, the cytotoxic effect of kunstlerone (1) was investigated by the addition of the MTT tetrazolium salt to various treated cancer cell lines. Taken together, the cytotoxic effects exerted by kunstlerone (1) as a promising compound and suggest its potential as anti proliferation agent. To our knowledge, the cytotoxic potentials of kunstlerone (1) have not been examined and the underlying molecular mechanisms remain to be discovered.

The Constituents of *Beilschmiedia kunstleri* grown in the Pahang, Malaysia was found to possess antimicrobial and cytotoxic activities. The crude methanol extract displays antigrowth activity and kunstlerone (1), isocaryachine (3) and noratherosperminine (8) displayed complete inhibition against *P. shigelloides*. In evaluation

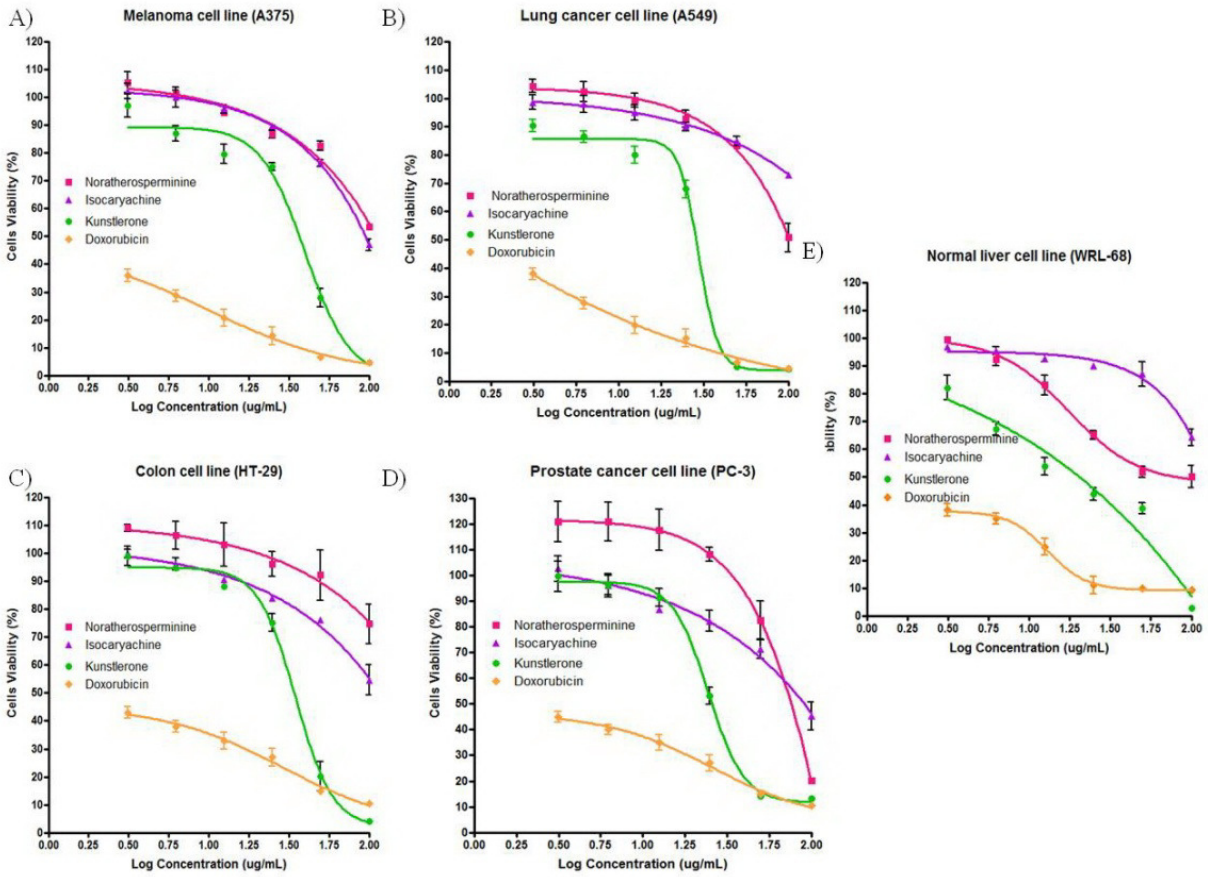

Figure 3. EC_{50} for the various compounds towards different cell lines.

Table 3. Results of tested with a series of different doses on A549, PC-3, A375, HT-29 and WRL-68, under determining EC_{50} for the test agents towards different cell lines.

		EC_{50} ± S.D (µg/mL)		
Cell line	**kunstlerone**	**isocaryachine**	**noratherosperminine**	**Doxorubicin**
A375	33.78	94.26	> 100	1.364
A549	28.02	> 100	> 100	1.550
HT-29	33.65	> 100	> 100	1.957
PC-3	26.78	91.54	72.55	2.125
WRL-68	16.46	> 100	72.37	1.731

ampicillin, a positive be in charge of this folder, showed antigrowth against *P. shigelloides*. Kunstlerone (1) showed also moderate cytotoxicity against various cancer cell lines such as A549, PC-3, A375, HT-29 and WRL-68. Therefore, the antimicrobial and cytotoxic activity study revealed that this plant has potential bioactivity.

Experimental

General

The optical rotations were recorded on a JASCO (Japan) P1020 Polarimeter equipped with a tungsten lamp; MeOH as solvent. The mass spectra were obtained from LCMS-IT-TOF, Shimadzu. The ultraviolet spectra were obtained in MeOH on a Shimadzu UV-310 ultraviolet-visible spectrometer. The Fourier Transform Infrared (FTIR) spectra were obtained with $CHCl_3$ (NaCl window technique) on a Perkin Elmer 2000 instrument. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in deuterated chloroform on a JEOL 400 MHz spectrometer; chemical shifts are reported in ppm on δ scale, and the coupling constants are given in Hz. Mayer's reagent was used for alkaloid screening. Aluminum TLC sheets and PTLC (20 × 20 cm Silica gel 60 F_{254}) were used in the TLC analysis.

The TLC and PTLC spots were visualized under UV light (254 and 366 nm) followed by spraying with Dragendorff's reagent for an alkaloid detection. All solvents, except those used for bulk extraction, were AR grade.

Plant materials

The leaves of *Beilschmiedia palembanica (Miq.)* Kosterm (KL 5373) was collected from Hutan Simpan Sungai Temau, Kuala Lipis, Pahang by L.E. Teo and Din. at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia and at the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and isolation of neolignan and the alkaloids

The air-dried leaves (2.00kg) of *Beilschmiedia palembanica* (Miq.) Kosterm was extracted exhaustively with hexane (12.0L) for 72 hours. The residual plant material was dried and left for 5 h after moistening with 10% NH_4OH. It was then macerated with CH_2Cl_2 (10.0L) for 6 days. After filtration, the supernatant was concentrated to 500 mL at room temperature (30°C) followed by acidic extraction with 5% HCl until a negative Mayer's test result was obtained. The aqueous solution was made alkaline to pH=11 with NH_4OH and re-extracted with CH_2Cl_2. This was followed by washing with distilled H_2O, dried over anhydrous sodium sulphate, and evaporation to give an alkaloid fraction (4.00g).

The extraction of alkaloids was repeated by using MeOH solvent and after acid base extraction obtained another (25.00 g) of crude alkaloid. The crude alkaloid (4.00g of CH_2Cl_2) was submitted to exhaustive column chromatography over silica gel (column dimension =2cm, length =75cm, silica gel 60, 70-230 mesh ASTM; Merck 7734) using CH_2Cl_2 gradually enriched with methanol (1% until 80% MeOH; volumes of eluent; 500mL were used for each percentage) to yield 6 fractions. Fraction 1 afforded one alkaloid as sebiferine (6) (19.00mg) (0.48%) (PTLC Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 99:1). Fractions 2, afforded three alkaloids identified as isocaryachine (3) (45.43 mg) (1.14%), salutarine (7) (26.80 mg) (0.66%) and *O*10-methylhernovine (9) (32.50 mg) (0.81%) (PTLC Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 98:2). Fractions 4 produced one benzamide as cinnamide (11) (21.20mg) (0.53%) (PTLC Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 95:5). Fraction 5 afforded two alkaloids as noratherosperminine (8), (36.30mg) (0.91%) and atherosperminine (10) (32.50mg) (0.81%) (PTLC Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 90:10). Fraction 6 produced two alkaloids as pseudocodamine (4) (24.33mg) (0.61%) and milonine (5) (34.32mg) (0.86%) using PTLC (Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 75:25.

Also, the crude alkaloid (5.00g of MeOH) was submitted to exhaustive column chromatography over silica gel (column dimension=3.00cm, length=75cm, silica gel 60, 70-230 mesh ASTM; Merck 7734) using CH_2Cl_2 gradually enriched with methanol to yield 5 fractions. Fraction 1 obtained norboldine (**2**) (18.66 mg) (0.47%) (PTLC Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 98:2).

Antioxidant assay

The free radical scavenging activity was determined using DPPH as described by Shimada *et al.* (Shimada et al., 1992). The DPPH radical scavenging activity assay is a decolorization assay that determines the activity of antioxidants to directly react with DPPH stable free radical by observing its absorbance at 517 nm with a spectrophotometer. A purple colored 1,1-diphenyl-2-picryl hydrazyl (DPPH), a stable free radical which is reduced to *α,α*-diphenyl-*β*-picryl hydrazine and give yellow color when reacts with antioxidant. The decolorization of purple color indicates the potential of antioxidants of the samples in which increased decolorization shows the higher scavenging activity of the samples.

Briefly, 0.1mM DPPH (1mL) dissolved in ethanol was added to an ethanol solution (3mL) of the tested compound at different concentrations (25, 50, 100, 150, 200µg/mL). An equal volume of ethanol was added in the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30min. Then the absorbance at 517nm was measured with a UV–VIS spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage of scavenging of DPPH was calculated using the following equation:

DPPH scavenging effect (%) = $\frac{A^{\circ}-A1}{A^{\circ}} \times 100$

where A^{o} is the absorbance of the control reaction and A1 is the absorbance in the presence of the sample.

CONCLUSIONS

(+)-Kunstlerone (1) as a neolignan, together with nine known alkaloids: (+)-norboldine (2), isocaryachine (3), pseudocodamine (4), milonine (5), sebiferine (6) and (-)-salutarine (7), noratherosperminine (8), 10-O-methyl hernovine (9), atherosperminine (10), and the amide was cinnamide (11), were isolated from the leaves of *Beilschmiedia Palembanica (Miq.) Kosterm.* The structures were established through various spectroscopic methods notably 1D- and 2D-NMR, UV, IR and LCMS-IT-TOF. (+)- Kunstlerone (1) showed a strong

antioxidant activity, with an SC_{50} of 20.0µg/mL. Additionally, the effect of compounds 1, 2 and 3 were evaluated on A549, PC-3, A375, HT-29 and WRL-68 cell lines. Kunstlerone 1 showed moderate cytotoxicity against various cancer cell lines such as A549, PC-3, A375, HT-29 and WRL-68, respectively with EC_{50} values of 28.02, 26.78, 33.78, 33.65 and 16.46µg/mL. The crude methanol extract showed antigrowth activity against *S. pyogenes* II and *B. cereus*, with MICs of 256µg/mL. The compounds kunstlerone (1), isocaryachine (3) and noratherosperminine (8) showed complete inhibition against *P. shigelloides*, with MIC ≤ 60 µg/mL compare to ampicillin, as a positive control, which showed antigrowth activity against *P. shigelloides* at MIC 10µg/mL.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support provided by University of Malaya Research Grant (UMRG 045/11BIO), Centre of Natural Products and Drugs Development (CENAR), Postgraduate Research Grant of University of Malaya (PS366/2010B) and High Impact Research (HIR) Grant of University of Malaya (F000009-21001). We also acknowledge the support provided by Faulty of science, Qom University of Technology, and Din Mat Nor and Rafly Syamsir for the plant samples and also Hairin Taha for editorial assistance.

REFERENCES

Barbosa FJM, Yoshida M, Gottlieb OR, Barbosa RCBC, Giesbrecht AM, Young CM (1987). Benzoyl esters and amides, styrylpyrones and neolignans from the fruits of *Aniba riparia*. *Phytochemistry*. *26*, 2615-2617.

Braga P, Dos Santos D, Silva MD, Vieira P, Fernandes J, Houghton P, Fang R (2007). In vitro cytotoxicity activity on several cancer cell lines of acridone alkaloids and N-phenylethyl-benzamide derivatives from Swinglea glutinosa (Bl.) Merr. *Nat Prod Res 21*, 47-55.

Chen JJ, Chou ET, Peng CF, Chen IS, Yang SZ, Huang HY (2007). Novel epoxyfuranoid lignans and antitubercular constituents from the leaves of *Beilschmiedia tsangii*. *Planta Med. 73*, 567-571.

Chouna JR, Nkeng-Efouet PA, Lenta BN, Devkota KP, Neumann B, Stammler HG, Kimbu SF, Sewald N (2009). Antibacterial endiandric acid derivatives from *Beilschmiedia anacardioides. Phytochemistry 70*, 684-688.

Corner EJH (1988). *Wayside Trees of Malaya*, 3rd ed.; Malaysian nature society: Kuala Lumpur, Malaysia.

Funasaki M, Lordello AL, Viana AM, Santa-Catarina C, Floh EIS, Yoshida M, Kato MJ (2009). Neolignans and sesquiterpenes from leaves and embryogenic cultures of *Ocotea catharinensis* (Lauraceae). *J. Braz. Chem. Soc*. *20*, 853-859.

Guinaudeau H, Leboeuf M, Cavé A (1979). Aporphine alkaloids. II. *J. Nat. Prod. 42*, 325-360.

Harborne JB, Méndez J (1969). Flavonoids of *Beilschmiedia miersii. Phytochemistry 8*, 763-764.

Kitagawa L, Minagawa K, Zhang RS, Hori K, Doi M, Inoue M, Ishida T, Kimura M, Uji T, Shibuya H (1993). Dehatrine, an antimalarial bisbenzylisoquinoline alkaloid from the Indonesian medicinal plant *Beilschmiedia madang,* isolated as a mixture of two rotational isomers. *Chem. Pharm. Bull. 41*, 997-999.

Lee JC,Kim HR, Kim J, Jang YS (2002). Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten. *J. Agric. Food. Chem. 50*, 6490-6496.

Lenta BN, Tantangmo F, Devkota KP, Wansi JD, Chouna JR, Soh RCF, Neumann B, Stammler HG, Tsamo E, Sewald N (2009). Bioactive Constituents of the Stem Bark of *Beilschmiedia zenkeri*. *J. Nat. Prod*., 72, 2130–2134.

Okusa P, Penge O, Devleeschouwer M, Duez P (2007). Direct and indirect antimicrobial effects and antioxidant activity of Cordia gilletii De Wild (Boraginaceae). *J. Ethnopharmacol. 112*, 476-481.

Pudjiastuti P. Mukhtar MR, Hadi AHA, Saidi N, Morita H, Litaudon M, Awang K (2010). (6, 7-Dimethoxy-4-methylisoquinolinyl)-(4´-methoxyphenyl)-methanone, a new benzylisoquinoline alkaloid from *Beilschmiedia brevipes*. *Molecules 15*, 2339-2346.

Rachmatiah T, Mukhtar MR, Nafiah MA, Hanafi M, Kosela S, Morita H, Litaudon M, Awang K, Omar H, Hamid AHA (2009). (+)-*N*-(2-Hydroxypropyl)lindcarpine: A new cytotoxic aporphine isolated from *Actinodaphne pruinosa* Nees. *Molecules 14*, 2850-2856.

Ribeiro AB, Bolzani VS, Yoshida M, Santos LS, Eberlin MN, Silva DHS (2005). A new neolignan and antioxidant phenols from *Nectandra grandiflora*. *J. Braz. Chem. Soc. 16*, 526-530.

Rossi MH, Yoshida M, Maia JGS (1997). Neolignans, styrylpyrones and flavonoids from an *Aniba* species. *Phytochemistry 45*, 1263-1269.

Setzer WN,Haber WA (2007). Leaf essential oil composition of five species of *Beilschmiedia* from Monteverde, Costa Rica. *Nat. Prod. Commun. 2*, 79-83.

Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem. 40*, 945-948.

Soepadmo E (1999). *Phytochemicals and Biopharmaceuticals from the Malaysian Rain Forest, Forest Research, Botanical Study of Malaysian Medicinal Plant and Appraisal*; Kepong: Kuala Lumpur, Malaysia, p.10.

Tchiegang C, Parmentier M (2008). Chemical composition and nutritional evaluation of two Cameroonian soup thickeners: *Belschmiedia Jacques felexii* and *Belschmiedia anacardiodes. Int. J. Food Sci. Technol. 45*, 187-189.

Yang PS, Cheng MJ, Chen JJ, Chen IS (2008). Two new endiandric acid analogs, a new benzopyran and a new benzenoid from the root of *Beilschmiedia erythrophloia*. *Helv. Chim. Acta. 91*, 2130-2138.

Yang PS, Cheng MJ, Peng CF, Chen JJ, Chen IS (2009). Endiandric acid analogs from the roots of *Beilschmiedia erythrophloia*. *J. Nat. Prod. 72*, 53-58.