BRJ BASIC RESEARCH JOURNALS

Basic Research Journal of Microbiology ISSN 2354-4082 Vol. 6(1) pp. 08-15 November 2019
Available online http//www.basicresearchjournals.org

Full Length Research Paper

Phytochemical compounds from leaves and bark of *Beilschmiedia Kunstleri* gamble

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Acceptance 11 November, 2019

ABSTRACT

The phytochemical studies on one species of Lauraceae family; *Beilschmiedia kunstleri* Gamble have been performed. The phytochemical study involves extraction using Soxhlet extractor, isolation by using various chromatographic methods such as column chromatography, thin layer chromatography and higher performance liquid chromatography and the structural elucidation of the isolated compounds was established on the basis of extensive spectroscopic studies including UV, IR, CD, OR, 1D-NMR (^{1}H-NMR, ^{13}C-NMR), 2D-NMR (^{1}H-^{1}H COSY, NOESY, HMQC/HSQC and HMBC) and MS. Seven compounds which consist of six alkaloids, in which four aporphines, one benzylisoquinoline and one morphinandienone, and one neolignan were isolated from the leaves of *Beilschmiedia kunstleri* Gamble, whereas the bark yielded nine compounds which consist of seven alkaloids, one neolignan and one dienamide.

Keywords: beilschmiedia; lauraceae; alkaloid; aporphines; chromatography; phytochemical.

INTRODUCTION

There are more than 2500 species belonging to the Lauraceae family all over the world, spread within the subtropics and tropics of eastern Asia and South and North America Simie et al., (2004). Many plants of Lauraceae have been affianced in folk medicine for their attractive bioactivities. For illustration, Beilschmiedia species is a source which can be treat arthritis, rheumatism, etc. (Pratiwi et al., 2010; Bruno ep al., 2009; Emma et al., 2010; Lee and Shibamoto, 2002; Khan et al., 2003; Dyke, 1964). The bark of *Cinnamomum cassia* Blume is a very well-known traditional medicine that has been extensively used in Asian countries. The chemical extracts from *C. cassia* have been claimed to cut back on inflammation Lee and Shibamoto (2002), and to trim down serum glucose and full amount cholesterol Khan et al., (2003).

In the relations of Lauraceae, most of the alkaloids are in the right place to the isoquinoline class Dyke (1964). Lauraceae family is generally taking place in Southeast Asia and tropical America with 40 genera and over 2000 species Nordin and Rohaya (2006). In Malaysia, its contribution is about 213 species, from 16

genera Guinaudeau et al., (1979). Aporphine alkaloids are collectively in presence but there are also a small number of indole alkaloids and quinoline alkaloids Harborne and Mendez (1969). This genus is over and above known to formulate a large number of biologically active compounds demodulating attractive skeletons (Harborne and Mendez, 1969; Yang et al., 2009; Yang et al., 2008). The lignans and neolignans consist of a class of natural plant products which are consequential from cinnamic acid derivatives and which are related biochemically to phenylalanine metabolism, Sovová et al., (2007); David and Paul, (1984) in addition of alkaloids, flavonoids, and endiandric acid derivatives, lignans, neolignans and benzamides extracted and recognized by the Beilschmiedia species Mariko et al., (2009). Furthermore, this species has been the subject of a number of encyclopedic articles. Harborne and Mendez, 1969; Yang et al., 2009; Chouna et al., (2009). Moreover, some of compounds such as the endiandric acid derivatives and Epoxyfuranoid lignans have shown strong antibacterial results and antitubercular activities Yang et al., (2008); Engler et al., (1993); Engler et a;., (1994).

Experimental

General experimental procedures

The optical rotations were recorded on a Jasco (Japan) P-1020 Polari meter equipped with a Sodium lamp; MeOH as solvent. LC-MS were obtained on an Agilent Technologies 6530 Accurate-Mass Q-TOF LC-MS. 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 1H-NMR and ^{13}C-NMR spectra were recorded in Deuterated Chloroform on a JEOL 400 MHz (unless stated otherwise) instrument; chemical shifts are reported in ppm on 8 scale, and the coupling constants are given in Hz. Silica gel 60, 70-230 mesh ASTM (Merck 7734) was used for column chromatography. Mayer's reagent was used for alkaloid screening. TLC Aluminum sheets and PTLC (20×20cm Silica gel 60 F254) were used in the TLC analysis. The TLC and PTLC spots were visualized under UV light (254 and 366nm) followed by spraying with Dragendorff's reagent for an alkaloid detection. All solvents, except those used for bulk extraction are AR grade.

Plant Material

Beilschmiedia kunstleri Gamble (Lauraceae), collected from Hutan Simpan sungai Tekam, Jerantut, Pahang, Malaysia was identified by Mr. Teo Leong Eng. A voucher specimen (KL 5627) is deposited 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. *Beilschmiedia kunstleri* Gamble is a tall tree up to 12m tall and 14cm diameter, bark reddish brown, striated; inner bark pale brown; young shoots reddish brown; leaves simple, alternate or spinally arranged, coriaceous, from elliptic lance late to ablanceolate, apex blunt to rounded, base obtuse, 25-60cm × 10-18cm, bright green above, paler below, drying brown to deep brown, midrib raised above, secondary nerves 20 pairs, arching near the margin; P.T.O.

Natural product extraction and isolation

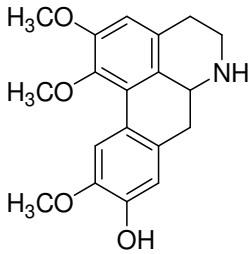
The air-dried leaves (2.50kg) of *Beilschmiedia kunstleri* Gamble were ground and extracted exhaustively with hexane (10.00 l) for 72 hours. The residual plant material was dried and left for 4 h after moistening with 10% NH_4OH. It was then macerated with CH_2Cl_2 (12.00 l) for 4 days. After filtration, 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). We repeat the extraction of alkaloids by using MeOH solvent and after acid base extraction obtained another (10.00g) of crude alkaloid. The crude alkaloid (4.00g of CH_2Cl_2 and 10.00g of MeOH) was submitted to exhaustive column chromatography over silica gel using CH_2Cl_2 gradually enriched with methanol to yield fractions. Six alkaloids, namely laurotetanine (1), boldine (2), norboldine (3), *N*-methylisococlaurine (4), cassythicine (5) and pallidine (6), and one neolignan namely kunstlerone (7) were isolated from the leaves of *Beilschmiedia kunstleri* Gamble.

Furthermore, air-dried bark (1.50kg) of Beilschmiedia kunstleri Gamble were ground and extracted exhaustively with hexane (5.00 l) for 72hours. The residual plant material was dried and left for 5h after moistening with 10% NH_4OH. It was then macerated with CH_2Cl_2 (9.00 l) for 3 days. After filtration, the

Table 1: Chemical constituent from *Beilschmiedia kunstleri* Gamble

Species	Plant Part	Remark	Type of skeleton
Beilschmiedia kunstleri Gamble	Leaves	laurotetanine (1)	aporphine
		Boldine(2)	aporphine
		norboldine(3)	aporphine
		N-methylisococlaurine(4)	benzylisoquinoline
		cassythicine (5)	aporphine
		pallidine(6)	morphinandienone
		kunstlerone (7)	neolignan
		kunstlerone(7)	neolignan
		3, 4-dimethoxy-1-phenanthreneethanamine (8)	aporphine
		isocaryachine(9)	pavine
	Bark	noratherosperminine(10)	phenanthrene
		N-demethylphyllocryptine (11)	benzylisoquinoline
		laurotetanine(1)	aporphine
		boldine (2)	aporphine
		cassythicine(5)	aporphine
		kunstleramide(12)	dienamide

Figure 1. Chemical structurs of constituents of leaves and bark (figure 1-12)



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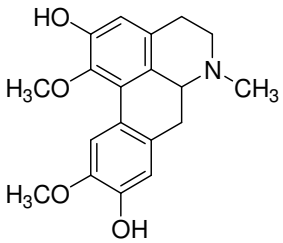

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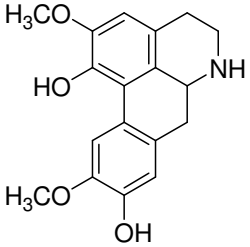

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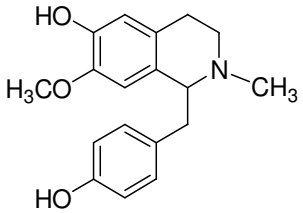

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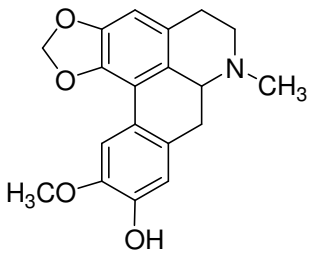

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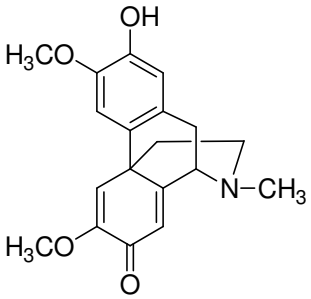

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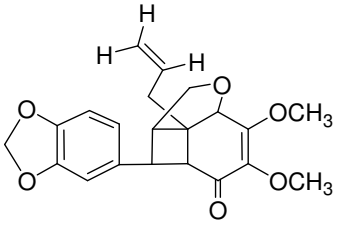

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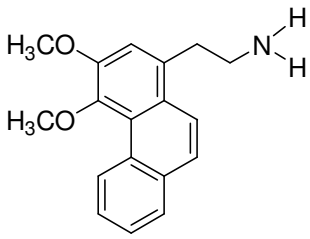

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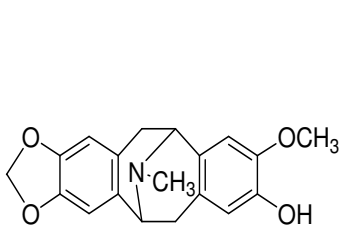
supernatant was concentrated to 500mL at room temperature (30°C) followed by acidic extraction with 5% HCl until a negative Mayer's test result was obtained. The aqueous solution was made alkaline to pH 11 with NH_4OH and re-extracted with CH_2Cl_2. This was followed by washing with distilled H_2O, dried over anhydrous sodium sulphate, and evaporation to give an alkaloid fraction (2.90g). We repeat the extraction of alkaloids by using MeOH solvent and after acid base extraction obtained another (35.00g) of crude alkaloid. The crude alkaloid from CH_2Cl_2 and MeOH was submitted to exhaustive column chromatography over silica gel using CH_2Cl_2 gradually enriched with methanol to yield fractions. These Fractions afforded Seven



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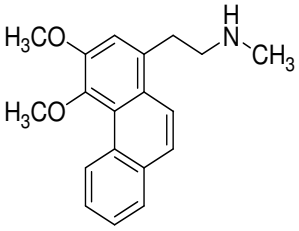

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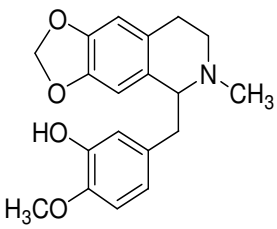

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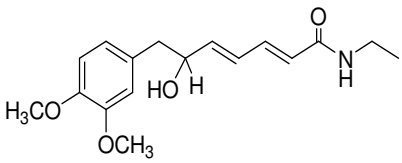

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alkaloids, one new neolignan, and one new dienamide; kunstleramide were isolated from the bark of *Beilschmiedia kunstleri* Gamble. The alkaloids belong to three aporphines, one benzylisoquinoline, two phenanthrenes and one pavine. The neolignan was kunstlerone (7) and it was also present in the leaves. The isolated alkaloids were 3, 4-dimethoxy-1-phenanthreneethanamine (8), isocaryachine (9), noratherosperminine (10), *N*-demethylphyllocryptine (11), laurotetanine (1), cassythicine (5), boldine (2) and kunstleramide (12). Laurotetanine, cassythicine and boldine were also present in the leaves of *Beilschmiedia kunstleri.* (Table 1-12 above).

RESULTS AND DISCUSSION

In additional search for phytochemical compounds and interesting chemical entities from Malaysian flora, we have performed a phytochemical study on the leaves of a Malaysian Lauraceae, *Beilschmiedia kunstleri* Gamble, which has led to the isolation of one neolignan; kunstlerone (7) Mollataghi et al., (2011) and six known alkaloids: norboldine (3) Sulaiman et al., (2011), N-methylisococlaurine (4) Yuan et al., (2006), cassythicine (5) Gözler et al., (1990), laurotetanine (1) Babcock and Segelman (1974), boldine (2) Rachmatiah et al., (2009) and pallidine (6) GuinaudeauH et al., (1983) (Figure 1). These alkaloids were obtained from the CH_2Cl_2 and CH_3OH extract of the leaves of *Beilschmiedia kunstleri* Gamble. Moreover, one neolignan same as neolignan of leaves extract; kunstlerone (7), seven known alkaloids from various types of alkaloids, 3, 4-dimethoxy-1-phenanthreneethanamine (8) Costa et al., (2010), isocaryachine (9) Chen et al., (1979), noratherosperminine(10) Guinaudeau et al., 1988, N-demethylphyllocryptine (11) Guinaudeau et al., 1988, laurotetanine (1), cassythicine (5) and boldine (2) (Figure 1) and kunstleramide (12) were isolated from the bark of this species. Isolation from the bark of *B. kunstleri* Gamble yielded seven alkaloids; five aporphines, one benzylisoquinoline and one pavine. These compounds were obtained from the CH_2Cl_2 and CH_3OH extract of the bark of *B. kunstleri*.

Laurotetanine (1)

laurotetanine (4H-dibenzoquinolin-9-ol, 5, 6, 6a, 7-tetrahydro-1, 2, 10-trimethoxy-) as an alkaloid; [α] $\frac{25}{D}$ = +1.96 (30.50×10^{-4} M, MeOH) was afforded as a dark brown amorphous solid. The UV spectrum showed absorptions at 217 and 242 nm, a characteristic values for 1, 2, 9, 10-tetradisubstituted aporphine. The IR spectrum showed absorption peak at 3385 cm^{-1} indicated the presence of hydroxyl group and NH stretching in the structure. The LC-MS spectrum showed an intense pseudo molecular ion peak, $[M+H]^+$ at m/z 328.1527 corresponding to the molecular formula of $C_{19}H_{21}NO_4$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 6.57 (1H, *s*, H-3), 2.74 (1H, *dd*, *J*= 13.68, 4.64Hz, H-4), 3.01(1H, *dd*, *J*= 12.92, 4.1Hz, H-5$_\alpha$), 3.65 (1H, *m*, H-5$_\beta$), 3.80 (1H, *dd*, *J*= 4.40, 13.20Hz, H-6a), 2.64 (1H, *d*, *J*= 13.68, H-7), 6.77 (1H, *s*, H-8), 8.06 (1H, *s*, H-11), 3.64 (1H, *s*, 1-OCH_3), 3.86 (2H, *s*, 2-OCH_3), 3.87 (2H, *s*, 10-OCH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 144.3 (C-1), 126.8 (C-1a), 127.4 (C-1b), 152.2 (C-2), 110.8 (C-3), 129.0 (C-3a), 29.0 (C-4), 43.1 (C-5), 53.7 (C-6a), 36.5 (C-7), 129.7 (C-7a), 113.9 (C-8), 145.3 (C-9), 144.9 (C-10), 111.3 (C-11), 124.0 (C-11a), 60.2 (1-OCH_3), 56.1 (2-OCH_3), 55.9 (10-OCH_3).

Boldine(2)

This Alkaloid, boldine or 1, 10-dimethoxyaporphine-2,9-diol or (4H-dibenzoquinoline-2, 9-diol, 5, 6, 6a, 7-tetrahydro-1, 10-dimethoxy-6-methyl-) or 2, 9-dihydroxy-1, 10-dimethoxy-aporphine; [α] $\frac{25}{D}$ =+ 6.95 (3.4×10^{-4} M, MeOH) was isolated as a brownish amorphous and its UV spectrum showed absorptions at 222 and 280 nm indicated that these were aporphine substituted at positions 1, 2, 9 and 10. The IR spectrum

showed a broad band of hydroxyl stretching absorption at 3391 cm^{-1}. The mass spectrum of Boldine showed the molecular ion peak $[M+H]^+$ at m/z 328.1536 suggesting a molecular formula of $C_{19}H_{21}NO_4$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 6.64 (H, *s*, H-3), 2.59-3.13 (aliphatic protons, m, H-4, H-5, H-6a, H-7), 2.55 (3H, *s*, N-CH_3), 6.83 (H, *s*, H-8), 3.57 (3H, *s*, 1-OCH_3), 3.92 (3H, *s*, 10-OCH_3), 7.89 (H, *s*, H-11) ^{13}C-NMR ($CDCl_3$) δ ppm: 144.2 (C-1), 126.7(C-1a), 127.4 (C-1b), 148.9 (C-2), 110.1 (C-3), 128.9 (C-3a), 14.5 (C-4), 43.2 (C-5), 42.2 (N-CH_3), 56.2 (C-6a), 29.8 (C-7), 130.0 (C-7a), 113.2 (C-8), 145.3 (C-9), 144.9 (C-10), 60.4 (10-OCH_3), 60.0 (1-OCH_3), 123.9 (C-11a), 114.3 (C-11).

Norboldine(3)

Alkaloid, norboldine or laurelliptine or norisoboldine (4H-dibenzoquinoline-1, 9-diol, 5, 6, 64H-dibenzoquinoline-1, 9-diol, 5, 6, 6a, 7-tetrahydro-2, 10-dimethoxy- a, 7-tetrahydro-2, 10-dimethoxy-); $[\alpha]_D^{25}$ =+9.87 (38.50×10^{-4} M, MeOH), was isolated as a brown amorphous solid. The UV spectrum showed absorption band at 276 and 317 nm due to the degree of resonance in the biphenyl system and any bands in the region above 305 nm eliminated the possibility of a 9, 10-disubstitution. The IR spectrum showed broad band at 3432 cm^{-1} due to the presence of OH and NH functional groups. This alkaloid showed an $[M+H]^+$ at *m/z* 314.1397 suggesting a molecular formula of $C_{18}H_{19}NO_4$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 6.58 (1H, *s*, H-3), 2.68 (1H, *m* , 4-α), 2.91 (1H, *m*, 4-β), 2.94 (1H, *m* , 5-α), 3.29 (1H, *m*, 5-β), 3.72 (1H, *dd*, *J*= 13.12, 4.20Hz, H-6a), 2.60 (1H, *m*, H-7), 6.74 (1H, *s*, H-8), 3.29 (3H, *s*, 2-OCH_3), 3.72 (3H, *s*, 10-OCH_3), 7.84 (1H, s, H-11). ^{13}C-NMR ($CDCl_3$) δ ppm: 148.1 (C-1), 125.6 (C-1a), 128.0 (C-1b), 141.9 (C-2), 113.7 (C-3), 130.2 (C-3a), 130.1 (C-7a), 29.0 (C-4), 43.3 (C-5), 53.8 (C-6a), 36.7 (C-7), 114.1 (C-8), 145.1 (C-9), 145.6 (C10), 60.4 (2-OCH_3), 56.2 (10-OCH_3), 110.2 (C-11), 123.7 (C-11a).

N-methylisococlaurine(4)

N-methylisococlaurine (6-isoquinolinol, 1, 2, 3, 4-tetrahydro-1-[(4-hydroxyphenyl) methyl]-7-methoxy-2-methyl-) as an alkaloid; [α] $^{25}_{D}$ = +0.32 (31.70×10^{-4} M, MeOH) was afforded as a brown amorphous solid. The UV spectrum showed absorption band at 225 and 289 nm which were a characteristic of a benzylisoquinoline alkaloid. The IR spectrum showed absorption at 3009 cm^{-1} indicated the stretching of hydroxyl group in the structure. The LC-MS spectrum gave a pseudomolecular ion peak, $[M+H]^+$ at m/z 300.1643 consistent with the molecular formula of $C_{18}H_{21}NO_3$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 3.63-3.81 (1H, *m*, H-1), 3.63-3.81 (2H, *m*, H-3), 2.58-2.84 (2H, *m*, H-4), 6.53 (1H, *s*, H-5), 6.41 (1H, *s*, H-8), 2.78-3.00(2H, *m*, H-α), 6.63 (1H, *d*, *J*= 8.32Hz, H-2′),6.95 (1H, *d*, *J*= 8.32Hz, H-5′),6.95 (1H, *d*, *J* = 8.32, H-6′),6.63 (1H, *d*, *J*= 8.32Hz, H-2′), 3.81 (1H, *s*, 7-OCH_3), 2.45 (3H, *s*, N-CH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 64.8 (C-1), 46.4 (C-3), 24.8 (C-4), 126.9 (C-4a), 110.8 (C-5), 143.2 (C-6), 145.3 (C-7), 113.7 (C-8), 125.4 (C-8a), 40.6 (C-α), 131.4 (C-1′), 130.4 (C-2′), 115.2 (C-3′), 154.2 (C-4′), 115.2 (C-5′), 130.4 (C-6′), 55.8 (7-OCH_3), 42.2 (*N*-CH_3).

Cassythicine (5)

This alkaloid, cassythicine or *N*-methylactinodaphine; (5H-benzo-1, 3-benzodioxolo [6, 5, 4]quinolin-10-ol, 6, 7, 7a, 8-tetrahydro-11-methoxy-7-methyl-) with [α] $^{25}_{D}$ = +2.93(34.1×10^{-4} M, MeOH) was isolated as a brownish amorphous solid and its UV spectrum showed absorptions at 240 and 300 nm indicated that this was aporphine substituted at positions 1, 2, 9 and 10. The IR spectrum indicated the stretching of hydroxyl group by the absorption band at 3352 cm^{-1} and the absorption at 1093 and 942 cm^{-1} indicated the presence of methylenedioxy group. The LC-MS; HRESI (positive mode) mass spectrum revealed a pseudomolecular ion, $[M+H]^+$ at m/z 326.1391 (Figure 3.49), corresponding to the molecular formula of $C_{19}H_{19}NO_4$. ^{1}H-NMR ($CDCl_3$) δ, ppm:6.52 (1H, *s*, H-3), 2.67, 3.16 (2H, *m*, H-4), 2.71, 3.24 (2H, *m*, H-5), 3.64 (1H, *m*, H-6a), 2.74 (1H, *m*, H-7), 6.82 (1H, *s*, H-8), 7.63 (1H, *s*, H-11), 6.09, 5.93 (2H, *d*, *J*=1.45, 1.25Hz, OCH_2O), 3.98 (3H, *s*, 10-OCH_3), 2.61 (3H, *s*, N-CH_3) ^{13}C-NMR ($CDCl_3$) δ ppm:142.1 (C-1), 128.7 (C-1a), 125.9 (C-1b), 145.0 (C-2), 106.7 (C-3), 128.8 (C-3a), 116.9 (C-7a), 29.7 (C-4), 53.4 (C-5), 62.2 (C-6a), 33.2 (C-7), 114.4 (C-8), 146.9 (C-9), 145.5 (C-10), 56.1 (l0-OCH_3), 109.9 (C-11), 122.8 (C-11a), 100.4 (OCH_2O).

pallidine(6)

Alkaloid, pallidine; (*N*-methyl-2-hydroxy- 3, 6-dimethoxymorphinandien-7-one) with [α] $^{25}_{D}$ = -2.63 (7.6×10^{-4} M, MeOH) was isolated as yellow amorphous solid. The UV spectrum showed absorption bands at 243, 254, and 420 nm. The IR spectrum showed absorption band at 1644 cm^{-1}, characteristic of a cross conjugated cyclohexadienone system and the broad absorption band at 346cm^{-1} indicated the presence of

O-H stretch group. The LC-MS showed a molecular ion peak at *m/z* 328.1562 $[M+H]^+$ which corresponding to molecular formula of $C_{19}H_{21}NO_4$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 6.69 (1H, *s*, H-1), 6.76 (1H, *s*, H-4), 6.33 (1H, *s*, H-5), 6.31 (1H, *s*, H-8), 3.68 (1H, *d*, *J*=6.56Hz, H-9), 3.02 (1H, *dd*, *J*=6.19, 17.78Hz, H-10α), 3.47 (1H, *d*, *J*=17.11Hz, H-10β), 2.02 (1H, *m*, H-15), 2.95 (1H, *m*, H-16), 3.89 (3H, *s*, 3-OCH_3), 3.79 (3H, *s*, 6-OCH_3), 2.44 (3H, *s*, N-CH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 113.4 (C-1), 144.8 (C-2), 145.0 (C-3), 107.5 (C-4), 118.9 (C-5), 151.3 (C-6), 181.7 (C-7), 122.6 (C-8), 60.7 (C-9), 32.2 (C-10), 129.5 (C-11), 133.1 (C-12), 42.3 (C-13), 150.1 (C-14), 41.1 (C-15), 45.6 (C-16), 55.9 (3-OCH_3), 55.8 (6-OCH_3), 41.6 (*N*-CH_3).

Kunstlerone(7)

This Neolignan was identified as 3, 4-dimethoxy-3´, 4´-methylenedioxy-2, 9-epoxy-6, 7-cyclo-1, 8-neolign-11-en-5(5H)-one, named as (+)-kunstlerone; $[\alpha]_D^{25}$ = +123.11° (3.76×10^{-3} M, MeOH), was isolated as a white amorphous solid; UV max (MeOH): 405 nm and 303. The IR bands (KBr): 1725, 1654 and 1038 cm^{-1}. The IR spectrum revealed absorption band at 1725 cm^{-1} due to the C=O stretching vibration. ^{1}H-NMR ($CDCl_3$) δ, ppm: 6.80 (1H, *s*, H-2´), 6.76 (1H, *d*, *J*= 8.1Hz, H-5´), 6.71 (1H, *d*, *J*= 8.1Hz, H-6´), 4.32 (1H, *s*, H-2), 2.90 (1H, *d*, *J*= 8.5Hz, H-6), 3.21 (1H, *dd*, *J*= 7.5, 7.8Hz, H-7), 2.83 (1H, *m*, H-8), 3.72, 3.90 (2H, *dd*, *J*= 9.5, 3.9Hz, H-9) (*d*, *J*= 9.5Hz), 2.38 (1H, *m*, H-10), 5.66 (1H, *m*, H-11), 5.13, 5.12 (2H, *d*, J_{cis}=11, 5.11Hz, J_{trans}=16.5 Hz, H-12), 5.91 (1H, *s*, OCH_2O), 4.08 (1H, *s*, 3-OCH_3), 3.69 (1H, *s*, 4-OCH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 132.1 (C-1´), 107.2 (C-2´), 147.9 (C-3´), 146.3 (C-4´), 108.3 (C-5´), 119.6 (C-6´), 46.0 (C-1), 79.1 (C-2), 159.4 (C-3), 138.4 (C-4), 193.1 (C-5), 50.1 (C-6), 44.5 (C-7), 49.6 (C-8), 70.8 (C-9), 41.7 (C-10), 136.5 (C-11), 119.4 (C-12), 101.0 (OCH_2O), 58.4 (3-OCH_3), 60.5 (4-OCH_3).

3, 4-dimethoxy-1-phenanthreneethanamine (8)

Alkaloid: 3, 4-dimethoxy-1-phenanthreneethanamine was afforded as a white amorphous solid [94]. The UV spectrum exhibited absorptions at 223, 254 and 283 nm. The IR spectrum showed absorption peak at 3341.43 cm^{-1} indicated the presence of amine group and the absorption at 2849 and 1600 cm^{-1} indicated the presence of aromatic ring in the structure. The LC-MS; EIS (positive mode) spectrum showed an intense pseudomolecular ion peak, $[M+H]^+$ at m/z 282.1525 corresponding to the molecular formula of $C_{18}H_{19}NO_2$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 7.30 (1H, *s*, H-2), 9.64 (1H, *dd,* *J*=9.38, 1.75Hz, H-8), 7.62 (1H, *d,* *J*=9.30Hz, H-9), 7.85 (1H, *d,* *J*=9.05Hz, H-10), 3.73 (2H, *m,* H-11), 3.64 (2H, *m,* H-12), 4.01 (3H, *s*, 3-OCH_3), 3.90 (3H, *s*, 4-OCH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 125.9 (C-1), 132.8 (C-1a), 128.9 (C-1b), 114.9 (C-2), 151.0 (C-3), 146.4 (C-4), 130.0 (C-5), 126.8 (C-6), 126.8 (C-7), 126.1 (C-8), 130.3 (C-8a), 126.1 (C-9), 122.1 (C-10), 29.9 (C-11), 59.9 (C-12), 58.1 (3-OCH_3), 56.6 (4-OCH_3).

Isocaryachine(9)

Alkaloid: isocaryachine; (benzo[5,6]cycloocta[1,2]-1,3-benzodioxol-5,11-imin-8-ol, 5, 6, 11, 12-tetrahydro-9-methoxy-14-methyl-) with $[\alpha]_D^{25}$ = **-**2.63 (7.6×10^{-4} M, MeOH) was obtained as a brown amorphous [91]. The UV spectrum exhibited maximum at 278 and 297 nm. The IR spectrum showed absorption at 3435 cm^{-1} indicating the presence of a hydroxyl group in the structure. The presence of a methylenedioxy group was proven by its characteristic absorption at 1229 and 925 cm^{-1} which indicated asymmetric O-C-O stretching. The ESI-MS (positive mode) spectrum exhibited a pseudomolecular ion peak, $[M+H]^+$ at m/z 326.1360 suggesting a molecular formula of $C_{19}H_{19}NO_4$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 6.51 (1H, *d*, *J* = 4.16Hz, H-1), 6.42 (1H, *s*, H-4), 2.49 (1H, *d*, *J*=16.08Hz, H-5α), 3.24-3.34 (1H, *m*, H-5β), 3.87 (1H, *d*, *J*=8.32Hz, H-6), 6.51 (1H, *d*, *J*=4.16Hz, H-7), 6.35 (1H, *s*, H-10), 2.49 (1H, *d*, *J*=16.08Hz, H-11α), 3.24-3.34 (1H, *m*, H-11β), 3.87 (1H, *d*, *J*=8.32Hz, H-12), 3.78 (1H, *s*, 8-OCH_3), 2.44 (1H, *s*, 6, 12-N-CH_3), 5.74 (2H, *d*, *J*= 1.2Hz, OCH_2O). ^{13}C-NMR ($CDCl_3$) δ ppm: 109.0 (C-1), 130.9 (C-1a), 145.7 (C-2), 146.0 (C-3), 108.3 (C-4), 124.3 (C-4a), 34.1 (C-5), 56.5 (C-6), 128.9 (C-6a), 106.8 (C-7), 144.1 (C-8), 145.0 (C-9), 114.2 (C-10), 124.7 (C-10a), 32.9 (C-11), 56.1 (C-12), 55.7 (8-OCH_3), 40.5 (6, 12-*N*CH_3), 100.3 (OCH_2O).

Noratherosperminine (10)

Alkaloid BKB22, noratherosperminine; 3, 4-dimethoxy-*N*-methyl-1-phenanthreneethanamine was afforded as a white amorphous solid. The UV spectrum exhibited absorptions at 257 nm. The IR spectrum showed absorption peak at 3363 cm^{-1} indicated the presence of amine group and the absorption at 2849 and 1512 cm^{-1} indicated the presence of aromatic ring in the structure. The LC-MS spectrum showed an intense pseudomolecular ion peak, $[M+H]^+$ at m/z 296.1636 corresponding to the molecular formula of $C_{19}H_{21}NO_2$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 7.30 (1H, *s*, H-2), 9.66 (1H, *d,* *J*=9.23Hz, H-5), 7.63 (1H, *m,* H-6), 7.60 (1H, *m,* H-7),

7.83 (1H, *dd, J*=9.34Hz, 1.86, H-8), 7.85 (1H, *d, J*=9.33Hz, H-9), 7.90 (1H, *d, J*=9.43Hz, H-10), 3.36 (2H, *m,* H-11), 2.99 (2H, *m,* H-12), 4.03 (3H, *s*, 3-OCH_3), 3.89(3H, *s*, 4-OCH_3), 2.47 (3H, *s*, *N*-CH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 125.3 (C-1), 132.8 (C-1a), 128.3 (C-1b), 114.9 (C-2), 150.9 (C-3), 146.4 (C-4), 130.1 (C-5), 128.3 (C-5a), 126.7 (C-6), 126.7 (C-7), 126.6 (C-8), 101.2 (C-8a), 126.6 (C-9), 121.9 (C-10), 32.0 (C-11), 60.0 (C-12), 59.8 (3-OCH_3), 56.7 (4-OCH_3), 51.4 (*N*-CH_3).

N-demethylphyllocryptine (11)

N-demethylphyllocryptine as an alkaloid; phenol, 2-methoxy-5-[(5, 6, 7, 8-tetrahydro-6-methyl-1,3-dioxolo[4,5]isoquinolin-5-yl)methyl]- with optical rotation $[\alpha]_D^{25}$ = +2.92° (34.30×10^{-4} M, MeOH), was isolated as a dark brown amorphous. The UV spectrum showed absorption band at 241, 250, 270 and 285 nm which was a characteristic of a benzylisoquinoline alkaloid. The IR spectrum gave a broad band at 3419.45 cm^{-1} due to the presence of hydroxyl group in the structure. The LC-MS spectrum as ESI$^+$ indicated the pseudomolecular ion, $[M+H]^+$ peak at m/z 328.1578 corresponding to a molecular formula of $C_{19}H_{21}NO_4$. ^{1}H-NMR ($CDCl_3$) δ, ppm: 3.66 (1H, *m*, H-1), 2.72-3.18 (1H, *m*, H-3), 2.57-2.83 (1H, *m*, H-4), 6.52 (1H, *s*, H-5), 6.23 (1H, *s*, H-8), 3.02 (1H, *m*, H-α), 6.75 (1H, *s*, H-2′), 6.73 (1H, *d*, *J*=8.08Hz, H-5′), 6.57 (1H, *d*, *J*=8.28Hz, H-6′), 5.84 (2H, *d*, *J*=1.44Hz, OCH_2O), 3.85 (1H, *s*, 4′-OCH_3), 2.46 (1H, *s*, N-CH_3). ^{13}C-NMR ($CDCl_3$) δ ppm: 64.4 (C-1), 46.6 (C-3), 25.6 (C-4), 126.9 (C-4a), 108.3 (C-5), 145.42 (C-6), 145.4 (C-7), 107.9 (C-8), 130.1 (C-8a), 41.1 (C-α), 133.2 (C-1′), 115.6 (C-2′), 145.2 (C-3′), 145.1 (C-4′), 110.4 (C-5′), 120.9 (C-6′), 100.7 (OCH_2O), 56.0 (4′-OCH_3), 42.3 (*N*-CH_3).

Kunstleramide (12)

A dienamide; (2E, 4E)-7- (3′, 4′-dimethoxyphenyl)-*N*-ethyl-6- (*R*) - hydroxyhepta-2, 4-dienamide, named as (-)-kunstleramide with optical rotation $[\alpha]_D^{25}$ = -15.48° (*C*=4.2×10^{-2} M, MeOH), was obtained as a yellowish amorphous solid. The UV spectrum showed absorptions at UV max (MeOH): 301 (3.99) nm and 280 (3.99). The IR spectrum showed the presence of the absorption bands (KBr) at 1612 and 1659 cm^{-1} due to C=C and C=O, respectively and 3351 cm^{-1} due to N-H and O-H broad stretch absorption (overlapped), respectively. The LC-MS spectrum showed an intense pseudomolecular ion peak 328.1531 [M+Na$^+$] corresponding to the molecular formula of $C_{17}H_{23}NO_4$ (calc. 328.1525). ^{1}H-NMR ($CDCl_3$) δ, ppm: 5.74 (1H, *d*, *J*=15.0Hz, H-2), 7.15 (1H, *dd*, *J*=15.1, 8.9Hz, H-3), 6.26 (1H, *dd, J*=15.1, 11.2Hz, H-4), 6.06 (1H, *dd, J*=15.0, 5.1Hz, H-5), 4.37 (1H, *m*, H-6), 2.66 (H, *dd, J_β*=13.8, 7.04Hz, H-7), 2.80 (H, *dd, J_α*=13.1, 5.1Hz, H-7), 3.30 (2H, *q, J*=7.4 Hz, H-8), 1.10 (3H, *t, J*=7.4 Hz, H-9), 6.66 (1H, *d, J*=1.8Hz, H-2′), 6.76 (1H, *d*, *J*=8.2Hz, H-5′), 6.67 (1H, *dd*, *J*=8.2, 1.8Hz, H-6′), 3.81 (3H, *s*, 3′-OCH_3), 3.79 (3H, *s*, 4′-OCH_3), 5.43 (1H, *br s,* N-H) , 1.67 (1H, *br s,* O-H). ^{13}C-NMR ($CDCl_3$) δ ppm: 124.2 (C-2), 140.0 (C-3), 127.7 (C-4), 142.3 (C-5), 72.6 (C-6), 43.4 (C-7), 34.6 (C-8), 14.9 (C-9), 129.5 (C-1′), 112.6 (C-2′), 147.9 (C-3′), 149.0 (C-4′), 111.3 (C-5′), 121.6 (C-6′), 55.9 (3′-OCH_3), 55.9 (4′-OCH_3), 165.8 (C=O).

CONCLUSIONS

One plant species from the Lauraceae family, *Beilschmiedia kunstleri* Gamble was studied for their chemical constituents and bioactivity. *Beilschmiedia kunstleri* Gamble (KL 5627) was collected from Hutan Simpan Sungai Tekam, Jerantut, Pahang by L.E. Teo and Din. Phytochemical studies of this species from Lauraceae led to the identification of alkaloids as; aporphines and benzylisoquinolines, pavines, morphinandienones, phenanthrenes; one neolignan, one flavanone, one benzamides, one dienamide and one phenanthrenol which were isolated from the leaves and bark. The isolation of alkaloids from the leaves of *Beilschmiedia kunstleri* Gamble gave laurotetanine, *N*-methylisococlaurine, boldine, norboldine, cassythicine, pallidine and one new neolignan named as kunstlerone was also isolated from the leaves. The alkaloids, 3,4-dimethoxy-1-phenanthreneethanaminine, isocaryachine, noratherosperminine, *N*-dimethylphyllocryptine, cassythicine, boldine and laurotetanine were isolated from the bark of this species together with one new amide, kunstleramide.

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.

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