Iranian Journal of Organic Chemistry Vol. 5, No. 1 (2013) 989-993

Alkaloids of the bark *Lindera oxyphylla* and their bioactivity

Masoumeh Hosseinzadeh[a*] and Jamaludin Mohamad[b]

[a]*Centre for Natural Products and Drug Discovery, Block D Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur Malaysia.*
[b] *Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.*

Received: November 2012; Revised: January 2013; Accepted: January 2013

Abstract: Phytochemical analysis of the bark of *Lindera oxyphylla* yielded a five known alkaloids. The structures were determined on the basis of special studies of 1D and 2D NMR techniques. In addition, in this report, we investigated the anticancer effects and antioxidant activities of the five extracted compounds.

Keywords: *Lindera oxyphylla*, Alkaloid, Antioxidant, Anticancer.

Introduction

Lauraceae family is normally occurring in Southeast Asia and tropical America with 40 genera and over 2000 species [1, 2]. In Malaysia, its contribution is about 213 species, from 16 genera [2]. *Lindera oxyphylla* is belonging to a large Lauraceae family group that contains more alkaloids. Alkaloids have been reported to exhibit other multiple biological effects such as antiviral [3], antibacterial [4], anti-inflammatory [5, 6], vasodilator [7], anticancer [8] and anti-ischemic [9, 11].

We have performed a phytochemical study on the bark of Malaysian Lauraceae, *Lindera oxyphylla*, which led to the isolation of (+)-laurotetanine (**1**), *N*-Methyllaurotetanine (**2**), (+)-Norboldine(**3**), (+)-10-O-Methy-N-methyllhernovine(**4**) and (+)-Norisoboldine (**5**) Figure **1**. In this paper deals with the isolation, structural elucidation and the antioxidant activities of the compounds have been evaluated using the DPPH method and the results are given of the concentration of the sample decreasing 50% of free radical scavenging (IC_{50}),compound 3 showed IC_{50}=16.92 μM. The anticancer result showed compound **1** cell line A375 EC_{50} = 37.09, compound **2** A 549 EC_{50} = 17.34 and WRL-68 EC_{50} = 22.03, compound **3** A 549 EC_{50} = 65.03. Compound **4** A375 EC_{50} = 54.76 and compound **5** A375 EC_{50} = 12.57 and A 549EC_{50} = 23.56. and Anticancer activities using A549 (Non-small cell lung cancer), A375 (Melanoma), WRL-68 (Normal hepatic cell line).

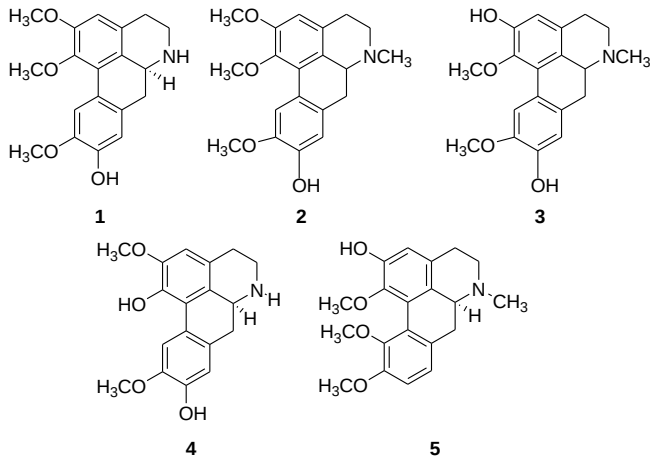


Figure 1: Chemical structure of compounds **1-5:**

Results and discussion

Compounds **1-5** were isolated from the bark of *Lindera oxyphylla*.

*Corresponding author. Tel/Fax: (+60) 172 835748, E-mail: ma_hosseinzadeh@yahoo.com

Alkaloid **1:** laurotetanine with IUPAC name 4H-dibenzoquinolin-9-ol, 5, 6, 6a,7-tetrahydro-1,2,10-trimethoxy with $[\alpha]_D^{25}$= +29.4º (2.00×10^{-4} g/100 mL, MeOH) was afforded as a dark brown amorphous solid [12]. The UV spectrum showed absorptions at λ_{max} (MeOH) nm (log Ɛ), 217 (2.345) and 242 (3.098) nm thus suggesting a 1, 2, 9, 10-tetrasubstituted aporphine skeleton. The IR spectrum showed absorption peak at 3429 cm^{-1} indicated presence of NH group and also hydroxyl group[13] . The LC-MS spectrum showed an intense pseudomolecular ion peak, $[M+H]^+$ at *m/z* 328.1566 corresponding to the molecular formula of $C_{19}H_{21}NO_4$.

Alkaloid **2:** *N*-methyllaurotetanine with $[\alpha]_D^{25}$=+15.0 ($2.\ 00\times10^{-4}$ g/100 mL , MeOH) was isolated as a brown amorphous solid and its UV spectrum showed absorptions at λ_{max} (MeOH) nm (log Ɛ) 222 (2.098), 280 (1.987) and 320 (3.564) nm [14] indicated the aporphine substituted at positions C-1, C-2, C-9 and C-10 (Shamma et al., 1964). The IR spectrum showed a broad band of hydroxyl absorption at 3391 cm^{-1}. The LC-MS revealed the precence a pseudomolecular ion peak, $[M]^+$ at *m/z* 401.1625 consistent with the molecular formula of $C_{24}H_{35}NO_4$

Alkaloid **3:** Norboldine with $[\alpha]_D^{25}$= +9.87 ($2.\ 00\times10^{-4}$ g/100 mL, MeOH), was isolated as a brown amorphous solid [15]. The UV spectrum showed absorptions λ_{max} (MeOH) nm (log Ɛ) at 276 (3.765) and 317 (2.987) nm [16]. The IR spectrum showed broad band at 3432 cm^{-1} due to the presence of OH and NH functional groups. The LC-MS revealed a pseudomolecular ion peak, $[M+H]^+$ at *m/z* 314.1397 suggesting a molecular formula of $C_{18}H_{19}NO_4$.

Alkaloid **4:** (+)-10-O-Methyl-N-methylhernovine with $[\alpha]_D^{25}$=+4.50 (2.00×10^{-4} g/100mL, MeOH) was afforded as a brownish amorphous powder. The UV spectrum showed absorptions at λ_{max} (MeOH) nm (log Ɛ) 244 (1.65) and 276 (2.76) nm, a characteristic values for 1, 2, 10, 11-tetrasubstituted aporphine. The IR spectrum showed absorption peak at 3390 cm^{-1} indicated the presence of hydroxyl group in the structure. The LC-MS spectrum showed an intense pseudomolecular ion peak at m/z 328.20 $[M+H]^+$ corresponding to the molecular formula of $C_{19}H_{21}NO_4$

Alkaloid **5:** (+)-norisoboldine with $[\alpha]_D^{25}$=+11.52 (2.00×10^{-4} g/100 mL, MeOH) was afforded as a brown amorphous solid. The UV spectrum showed absorptions at λ_{max} (MeOH) nm (log Ɛ) 241 (1.87), 250 (1.98) and 270 (2.98) nm, a characteristic values for 1, 2, 9, 10-tetrasubstituted aporphine. The IR spectrum showed absorption peak at 3436 cm^{-1} indicated the presence of hydroxyl group in the structure[12].The LC-MS spectrum showed an intense pseudomolecular ion peak $[M+H]^+$ at m/z 314.1399 corresponding to the molecular formula of $C_{18}H_{19}NO_4$.

Biological Activity:

Antioxidant:

The free radicals including the superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and lipid peroxide radicals have been implicated in a number of disease processes including asthma , cancer, cardiovascular disease, cataracts, diabetes, gastrointestinal inflammatory diseases, liver disease, macular degeneration, periodontal disease and other inflammatory processes [17]. These radical oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and as a result of increased exposure to environmental and or dietary xenobiotic.

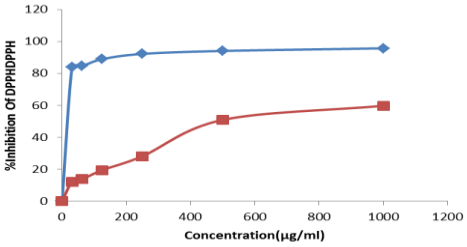
The antioxidants are the agents which can inhibit or delay the oxidation of an oxidisable substrate in a chain reaction. Antioxidant capacity assays may be broadly classified as electron transfer (ET) ¯ and hydrogen atom transfer (HAT) ¯based assays. There are several methods to measure total antioxidant activity of a compound or plant extract based on HAT, ET [18].

Free radical scavenging activity (DPPH):

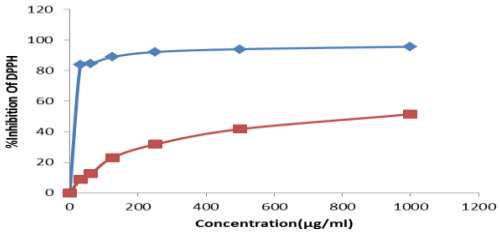
The model of scavenging the stable $DPPH^{\cdot}$ radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods [19]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [20]. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of $DPPH^{\cdot}$ radical caused by antioxidants because of the reaction between antioxidant molecules and the radical progresses. It is visually noticeable as a discoloration from purple to yellow. Hence, $DPPH^{\cdot}$ is usually used as a substrate to evaluate antioxidative activity of antioxidants [21].

An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system or to inhibit 50% of lipid peroxidation.

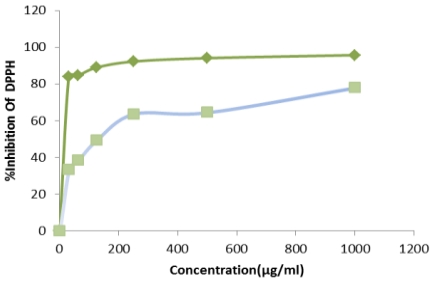
As shown in Figure **2**, the highest activity was observed in compound (**3)** due to its norboldine derivatives with hydroxyl group as electron donor reduced the DPPH radicals.



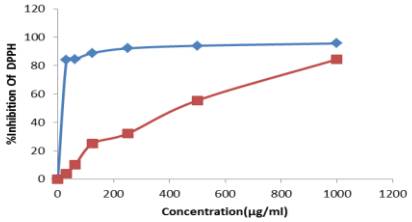
Compound 1:%IC_{50}=26.34



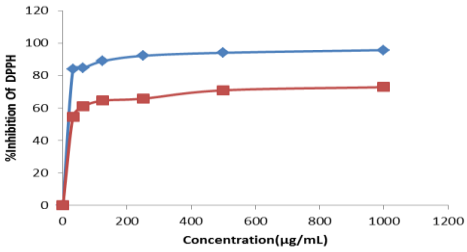
Compound 2:%IC_{50}=31.81



Compound **3**:%IC_{50}=16.92



Compound **4**:%IC_{50}=17.02



Compound **5**:%IC_{50}=20.77

Figure 2: Graph of concentration (µM) as % inhibition DPPH radical scavenging activity of alkaloids 1-5**.**

At the concentration of 200ug/ml, the DPPH radical inhibition of the compounds (**1 -5)** decreased in the following order: **3** (IC_{50} 16.92) > 4 (17.02) > **5** (20.77)> **1** (26.32) and **2**(IC_{50} 31.81)**.** Ascorbic acid (Vitamin C) a well-known antioxidant which is used as appositive controls shows 95% inhibition on DPPH radical at a concentration of 200ug/ml with IC_{50} 4.62±0.01.

Anti-cancer:

Cell culture:

All the cells that used 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 colon adenocarcinoma, MCF-7 human breast 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 cell line 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 determine the inhibitory effect of 6B, C3, D1, 34 and 44D on cell growth using the MTT assay. The MTT assay was *modified as described* by [22]. Briefly, cells were seeded at a density of 1 x 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 percentage) of absorbance of treated cells to untreated cells [23].

Statistical Analyses:

Each experiment was performed at least two times. Results are expressed as the means value ± standard deviation (SD). Log EC_{50} calculations were performed using the built-in algorithms for dose-response curves

with variable slope using Graphpad Prism software (version 4.0; GraphPad Software Inc., San Diego, CA). A fixed maximum value of the dose-response curve was set to the maximum obtained value for each drug.

Table 1: EC50 (μM) of five compounds of Lindera oxyphylla

Cell lines	1	2	3	4	5
A549	>100	17.34	65.03	>100	23.56
A375	37.09	>100	>100	54.76	12.57
WRL-68	>100	22.03	>100	>100	>100

Conclusion

Our current research led to the isolation 5 compounds from *Lindera oxyphylla* . Their structures were identified by 1D, 2D. The antioxidant result compounds **3** > **4** > **5** >**1** and **2**. The anticancer result showed compound **1** cell line A375 EC_{50} = 37.09, compound **2** A 549 EC_{50} = 17.34 and WRL-68 EC_{50} = 22.03, compound **3** A 549 EC_{50} = 65.03. Compound **4** A375 EC_{50} = 54.76 and compound **5** A375 EC_{50} = 12.57 and A 549EC_{50} = 23.56.

Experimental

General:

The ^{1}H-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.

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. Silica gel 60, 70-230 mesh ASTM (Merck 7734) was used for column chromatography. TLC Aluminum 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). All solvents, except those used for bulk extraction are AR grade.

Plant Materials:

Lindera oxyphylla (Lauraceae), from Herbarium Specimen Numbers (KL 5359) was collected from Hutan simpan Ulu Muda, Baling, Kedah 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

Extraction and Isolation:

The dried bark (4 kg) of *Lindera oxyphylla* were ground and moistened with 10% NH_4OH and left for 4 hrs. before soaking with CH_2Cl_2 (12.0 L) for 4 days. After filtration, the supernatant was concentrated to 500 mL followed by acidic extraction with 5% HCl until a negative Mayer's test result was obtained. The aqueous solution was basified to pH 11 with NH_4OH and re-extracted with CH_2Cl_2. The CH_2Cl_2 extract was filtered, dried over anhydrous sodium sulphate, and evaporated to give crude alkaloid (2.0 g). The crude extract were subjected to exhaustive column chromatography over silica gel using dichloromethane/methanol as a solvent system whit ratio 100:0→0:100 to give 65 fractions. Fraction 15 to 20 were combined and the resulted compounds was separated using PTLC Merck KGaA silica gel 60 F_{254}; and CH_2Cl_2-MeOH; 98:2 as a solvent to afford (+)-laurotetanine (10.0 mg, 0.5%), N-methyllaurotetanine (5 mg, 0.25%), was obtained from fraction, 30 to 35 using CH_2Cl_2-MeOH; 97:3 as a solvent and (+)-norboldine (10.0 mg, 0.5%) was obtained from fraction 49 to 50 using CH_2Cl_2-MeOH; 95:5 as a solvent. (+)-10-O-N-methylhernovine (10.0 mg, 0.5%), was obtained from fraction 62 to 63 using CH_2Cl_2-MeOH; 92:8 as a solvent and (+)-norisoboldine (5.0 mg, 0.025%), was obtained from fraction 64 to 65 using CH_2Cl_2-MeOH; 92:8 as a solvent.

Determination DPPH radical scavenging activity [10]:

The DPPH scavenging activity To 1.25 ml of 60 μM DPPH in methanol, 250μL of each (31.25,62.5,125,250,1000 μM) sample was added, and decrease in the absorbance was monitored after 1 min and then left to stand at room temperature for 30 min in the dark,and its absorbance was read at 517 nm . The absorbance of a control (methanol instead of sample) was also recorded after 1 min the wavelength (A517 control). Therefore, the percentage of inhibition was calculated by.

$$\%\text{Inhibition} = \frac{\text{A517 (control) - A517 (sample)}}{\text{A517 (control)}} * 100$$

Ascorbic acid was used as positive control. Control is DPPH and methanol concentration instead sample.

Acknowledgement

The authors gratefully acknowledge the financial support provided by Ministry of Higher Education (HIR grant F00009-21001) and Postgraduate Research Grant of University of Malaya (PS265/2010B).

References

[1]Soepadmo, E. *Phytochemicals and Biopharmaceutins from the Malaysian Rain Forest, Forest Research, Botanical Study of Malaysian Medicinal Plant and Appraisal*, Institute of Malaysia, Kuala Lumpur **1999**, 10.
[2]Corner, E. J. H. *Wayside Trees of Malaya*, 3 ed., 1, Cambridge **1988**, 371-381.
[3]Weber, J. M.; Ruzindana-Umunyana, A.; Imbeault, L.; Sircar, S. *Antivir. Res.* **2003,** *58*, 167.
[4]Alvesalo, J.; Vuorela, H.; Tammela, P.; Leinonen, M.; Saikku, P.; Vuorela, P. *Biochem. Pharmacol.* **2006**: *71*, 735.
[5]Subarnas, A.; Wagner, H. *Phytomedicine*, **2000,** 7, 401.
[6]Widlansky, M. E.; Duffy, S. J.; Hamburg, N. M.; Gokce, N.; Warden, B. A.; Wiseman, S. *Free Radic. Biol. Med*. **2005,** *38*. 499.
[7]Calderone, V.; Chericoni, S.; Martinelli, C.; Testai, L.; Nardi, A.; Morelli, I. N*aunyn-Schmiedebergs Arch Pharmacol*. **2004**, *370*, 290.
[8]Formica, J. V.; Regelson, W. *Food Chem. Toxicol*. **1995**, *33*, 1061.
[9]Rump, A. F.; Schussler, M.; Acar, D.; Cordes, A.; Ratke, R.; Theisohn, M. *Gen. Pharmacol*. **1995,** *26*, 603.
[10]Duthie, G. G.; Duthie, S. J.; Kyle, J. A. M. *Nuter. Res. Rev*. **2000**, *13*, 79.
[11]Mladenka, P.; Zatioukalovs, L.; Filipssky, T.; Hrdina, R.; *Free Radic. Biol. Med*. **2010**, *15*, 963.
undergoing electron transfer reactions.
[12]Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod*. **1987**, *51*, 389.
[13]Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod*. **1994**, *57*, 1033.
[14]Goodwin, S.; Shoodery, J. N.; Johnson, L. F. Significance of the nonequivalence of methylenedioxyhydrogen nuclei in the nuclear magnetic resonance spectra of aporphine alkaloids, *Proceedings of the Chemical Society London*, **1958**, 306-307.
[15]Pachler, K. G. R. A., R. R.; Baarschers, W. H. *Tetrahedron*, **1965**, *21*, 2159.
[16]Sanster, A. W.; Stuart., K. L. *Chem. Rev.* **1965**, *65*, 69.
[17]Niki, E. N. *Food Chem.* **2000**. *50*, 323.
[18]Apak, R.; Güçlü, K.; Demirata, B.; Özyürek, M.; Esin Çelik, S.; Burcu Bektaşoğlu, K. I. B. *Assay. Molec.* **2007**, *12*, 1496.
[19]Baumann, J.; Wurn, G.; Bruchlausen, V. Prostaglandin synthetase inhibiting O2_d radical scavenging properties of some flavonoids and related phenolic compounds. Naunyn-Schmiedebergs Archives of Pharmacology **2002**, *308*, R27.
[20]Soares, J. R.; Dins, T. C. P.; Cunha, A. P.; Ameida, L. M. *Free Rad. Res.* **1997**, *26*, 469.
[21]Duh, P.D., Tu, Y.Y., Yen, G.C. Antioxidant activity of water extract of Harng Jyur (Chrysanthemum morifolium Ramat). Lebnesmittel- Wissenschaft und-Technologie **1999**; 32, 269–277.
[22]Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem.* **2005**, *53*, 1841.
[23]Mosmann, T. *J. Immunol. Meth*. **1983**, *65*, 55.