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Isolation and characterization of alkaloids from Lindera Oxyphylla

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Abstract: The compounds (+)-O10-methylhernovine (1) (+)-norboldine (2) were obtained from phytochemical investigation of plant of Lindera Oxyphylla, a well medicinal plant. The structures of these compounds were established with the aid of spectroscopic methods, including analysis by 2D NMR spectroscopy. Determination of various antioxidant activities (2, 2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), ferric reducing power (FRAP), ferrous ion chelating (FIC) of leaves and bark of Lindera Oxyphylla. Compounds1-5 significantly showed DPPH scavenging activity with IC50 values of 161.93 ± 0.004, 41.32 ± 0.005 (, respectively,and FRAP with IC50% values of 467 ±0.02 ,273.93±0.005 .

Keywords: Lindera Oxyphylla, Flavonoids, Linderones, FRAP, DPPH, FIC.

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]. This genus is known to produce a large number of biologically active compounds with interesting skeletons such as Antioxidant , antibacterial and antitubercular activities [2] .Lindera Oxyphylla belonging large Lauraceae family group that contain more flavonoids and linderone.Flavonoids (the term is derived from the Latin word “flavus” meaning yellow) are ubiquitous plant secondary products that are best known as the characteristic red, blue, and purple anthocyanin pigment of plant tissues [3]. The antioxidant capacities of many alkaliods are much stronger than those of vitamins C and E [4]. we have performed a phytochemical study on the leaves and bark of a Ma laysian Lauraceae, Lindera Oxyphylla, which has led to the isolation of (+)-O10-methylhernovine (1) (+)-norboldine (2) .

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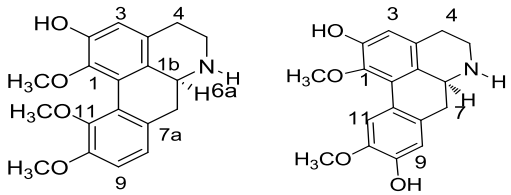

Figure 1. Structure of compounds 1-2.

Results and discussion

Biological Activity

Antioxidant

Antioxidants in biological systems have multiple functions which include protection from oxidative damage and in the major signaling pathways of cells. 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 [5]. DPPH, FRAP, Foline.Antioxidants have been shown to prevent the formation of reactive oxygen/nitrogen species through sequestering metal ions, directly reacting with and scavenging reactive oxygen or nitrogen species,

inhibiting oxidative enzymes (i.e. cyclooxygenases), as well as inducing antioxidant enzyme activities.

Free radical scavenging activity (DPPH):

The model of scavenging the stable DPPH· Radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods [6]. DPPH· Is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [7]. 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· 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· Is usually used as a substrate to evaluate antioxidative activity of antioxidants [8].

DPPH: $DPPH^{\cdot} + ArOH \rightarrow DPPH + ArO^{\cdot} + H+$

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.

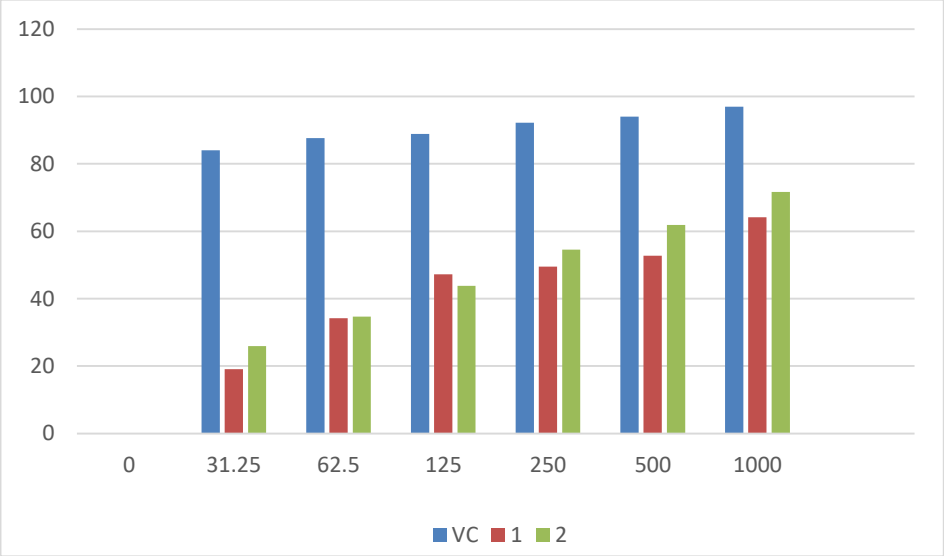

Figure 2. Proposed mechanism for the formation of **4**.

FRAP assay:

The ferric reducing-antioxidant power was measured accord into the method described by (8) with some modification. Generally induced by transition metal ions like Fe (III) in the presence of dissolved oxygen, gives rise to oxidative damage to lipids, and can be demonstrated by the following reactions [9]. The reducing properties of ferric ion are often used as an indicator of electron-donating activity.

$Fe(III) + ArOH \rightarrow Fe(II) + ArO^{\cdot} + H+$

$ArO^{\cdot} + LH \rightarrow ArOH + L^{\cdot}$

$L^{\cdot} + O2 \rightarrow LOO^{\cdot}$

$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$

$Fe(II) + LOOH \rightarrow Fe(III) + LO^{\cdot} + OH^{-}$

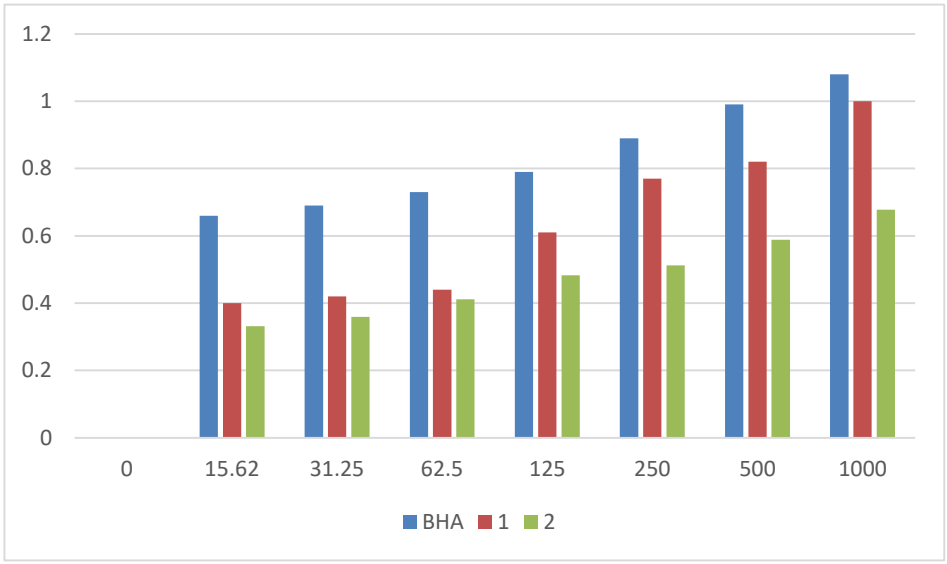

Figure 3. Ferric reducing-antioxidant power of the isolated compounds of *Lindera Oxyphylla*.

Metal chelating:

Ferrozine can quantitatively form complexes with Fe2+. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator [11]. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [12,13].

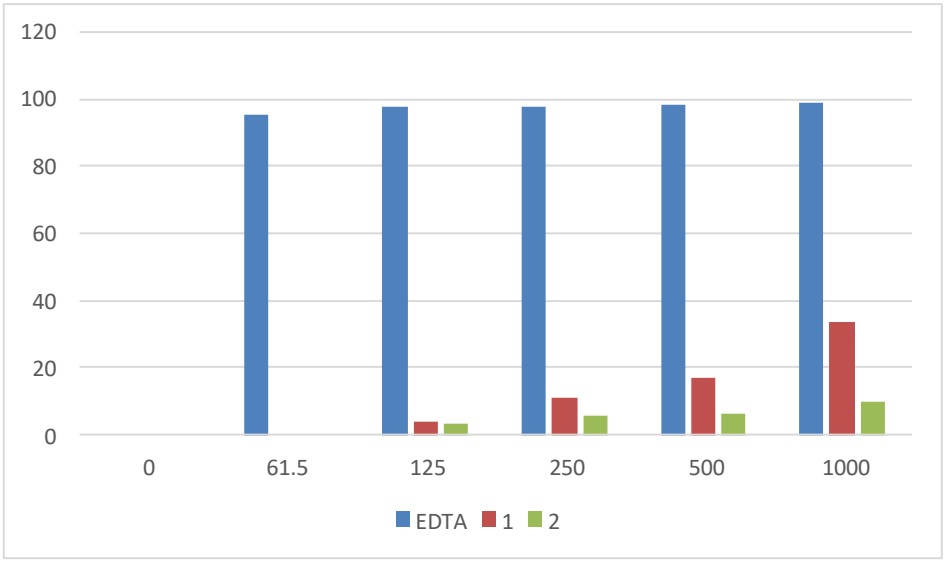

Figure 4. Cheleate effect of the isolated compounds of *Lindera Oxyphylla*.

Table 1: Antioxidant activities of five compounds of Lindera Oxyphyla using the DPPH assay, FRAP assay, and FIC assay.

Samples	IC50/DPPH (μg/ml) a	IC50/FRAP (μg/ml) b	IC50% FIC (μg/ml) c
1	161.93±0.004	476.0±0.02	Not found
2	41.320 ± 0.005	223.93±0.005	Not found
Vc	4.62±0.001	6.73±0.01	Not found
EDTA	-	-	9.69±0.01

Data are presented as the mean ± standard deviation (n=3). aThe antioxidant activity was evaluated as the concentration of the test sample required to decrease the absorbance at 517 nm by 50%. bThe antioxidant activity was evaluated as the concentration of the test sample needed to decrease the absorbance at 700 nm by 50%.c The antioxidant activity was valuated as the concentration of the test sample required to decrease the absorbance at 562 nm by50%.

Conclusion

Compounds **1** has been isolated as leaves and bark of Lindera Oxyphylla showed DPPH scavenging activity with $IC_{50=}8.5 \pm 0.004$. And FRAP with IC_{50}% values of 167.74±0.02.

Experimental

The optical rotations were recorded on a JASCO (Japan) P1020 Polarimeter equipped with a Tungsten lamp; MeOH as solvent and we have CD data on a spectrometer 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 ^{1}H-NMR and ^{13}C-NMR spectra were recorded in Deuterated Chloroform on a JEOL 400 MHz (unless stated otherwise) instrument; chemical shifts instrument JASCO (Japan) J-715 equipped with a tungsten lamp; MeOH as solvent. LC-MS were obtained on an Agilent Technologies 6530 Accurate-Mass Q-TOF 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. 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), collected from Hutan Simpan Sungai Tekam, Jerantut, Pahang, Malaysia was identified by Mr. Teo Leong Eng. A voucher specimen (KL5627) 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 of Alkoloids:

The dried bark (4 kg) of *Lindera Oxyphylla* were ground and extracted exhaustively with hexane for 72 hours after evaporation to give crude (13g). was submitted to exhaustive column chromatography over silica gel (column dimension =2cm, length =75cm, silica gel 60, 70-230 mesh ASTM; Merck 7734) using hexane gradually enriched with CH_2Cl_2 (1% until 80% CH_2Cl_2; volumes of eluent; 450mL were used for each percentage) to yield 30 fractions.. Fractions were then recombined on the basis of their TLC behavior to obtain 2 fractions. Fractions 10-20, afforded a alkolid as lindera oxyphylla(1)(%) (PTLC Merck KGaA silica gel 60 F_{254}; C_6H_{14}- CH_2Cl_2; 60:40) ,fractions 22-26 , afforded a Linderone as Lindera Oxyphylla (2)(%) (PTLC Merck KGaA silica gel 60 F_{254}; C_6H_{14}- CH_2Cl_2; 70:30).

Determination DPPH radical scavenging activity:

To 1.25 ml of 60 μM DPPH in methanol, 250 μL of each (31.25, 62.5, 125, 250, 1000 $\mu g/ml$) 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 [10]:

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

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

Antioxidant activity by ferric reducing antioxidant power (Frap):

The ferric reducing antioxidant power (FRAP). Potassium phosphate buffer (0.5 ml of 0.2 M and pH 6.6) and 0.5 ml of 1% w/v potassium ferric were mixed with 0.5 ml of sample of varying dilutions. The reaction mixture was incubated at 50 ^{C} for 20 min, after which 0.5 ml of 10% w/v trichloro acetic acid was added. The mixture was centrifuged at 3000 g for 10 min. (11-15) The supernatant solution (0.5ml) was mixed with Water (0.5 ml) and 0.1 ml of 0.1% w/v $FeCl_3$ to react for 10 min. Subsequently, the absorbance was measured at 700 nm. The IC_{50} value is the concentration of sample and the amount of gallic acid.

$$\% \text{ Effect} = \frac{\text{sample} - \text{control}}{\text{control}} \times 100$$

Antioxidant activity by Chelating effect on ferrous ions:

The chelating of ferrous ions of compounds was estimated by the method of (15-20). Briefly, different concentrations of methanol extract of compounds (125, 250, 500, 1000, μg/mL) were added to a solution of 2mM $FeCl_2$ (0.01mL). The reaction was initiated by adding 5mM ferrozine (0.02mL) and the mixture was shaken vigorously and left standing at room temperature for 10min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. All tests and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine–Fe2+ complex formation is given by this formula:

$$\% \text{ Inhibition} = \frac{A0(\text{control}) - A1(\text{sample})}{A0(\text{control})} \times 100$$

where A_0 was the absorbance of the control and A_1 the absorbance in the presence of the sample of compound and standards

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References

[1] Soepadmo, E. Phytochemicals and Biopharmaceutins from the Malaysian Rain Forest, Forest Research, Botanical Study of Malaysian Medicinal Plant and Appraisal, Institute Malaysia, Kuala Lumpur **1999**, 10.
[2] Corner, E. J. H. *Wayside Trees of Malaya, 3* ed., 1, Cambridge **1988**, 371.
[3] Formica, J. V.; Regelson, W. *Food Chem. Toxicol.* **1995**, *33*,1061
[4] Proir R. L.; Cao, G. *Hortic Sci.* **2000**; *35*, 588.
[5] Reşat, A.;Kubilay, G.; Birsen, D.; Mustafa, Ö.; Saliha, E.; *Molecules*, **2007**, *12*, 1496.
[6] Baumann, J.; Wurn, G.; Bruchlausen, V. *Naunyn-Schmiedebergs Archives of Pharmacology* **2002**, *308*, R27.
[7] Soares, J. R.; Dins, T .C. P.; Cunha, A.P.; Ameida, L. M. *Free Rad. Res.* **1997,** 26, 469.
[8] Duh, P. D.; Tu, Y. Y.; Yen, G. C. *Lebnesmittel-Wissenschaft und-Technologie* **1999**, *32*, 269.
[9] Huang, D.; Ou, B.; Prior, R.L. *J. Agric. Food Chem.* **2005**, *53*, 1841.
[10] Kwon, Y, L.; Vatten, D. V.; Shetty, K. *Pac. J. Clin.Nutr*.**2006**, *15*,107.
[11] Yamaguchi, F.; Ariga, T.; Yoshimira,Y.; Nakazawa, H. *J. Agric. Food Chemry.* **2000**, *48*, 180.
[12] Halliwell, B. Am. J. Medicine **1991,** *91*, 1422.
[13] Chang, L.W.; Yen, W.J.; Huang, S. C.; Duh, P.D. Food Chem. **2002**, *78*, 347.
[14] Mukherjee, R. K.; Fujimoto, Y.; Kakinuma, K. *Phytochemistry*, **1994**, *37*, 1641.
[15] Mulder, T. P.; Rietveld, A. G.; van Amelsvoort. J. M. *Am. J. Clin. Nutr.* **2005**, *81*, 256S.
[16] Nam, I.; Hyeyoung, K.; Youhui, L.; Jong Dae, P.; KyuSang, K.; Shin, K. *Planta Medica*, **1992**, *58*, 566.
[17] Napoli, C.; Lemieux, C.; Jorgensen, R. *Plant Cell* **1990**, *2*, 279.
[18] Natiello, M.; Contreras, R.; Facelli, J.; De Kowalewski, D. *J. Phys. Chem.* **1983**, *87*), 2603.
[19]Neacsu, Eklund, P. C.; Sjoholm, R. E,; Pietarinen, S. P.; Ahotupa, M. O.; Holmbom, B. R,; Willfor, S. M. *Eur. Aspen Holz. Roh. Werkst*, **2007**, *65*, 1.
[20] Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.*, **2003**, *66*, 1022.