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Biologically active compounds extracted from *lindera oxyphylla*

Masoumeh Hosseinzadeh[a*] and Nahid Sohrevardi[b]

[a]*Department of Chemistry, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran*
[b]*Department of Chemistry, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran*

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Abstract: The compounds [(2*S*)-5-hydroxy -6, 7-dimethoxy-2-phenyl chroman-4H-one] (**1**) 5, 7-dihydroxy -2(S)-phenyl chroman-4H-one (**2),**(*E*)-1-(2-hydroxy-4, 6-dimethoxyphenyl)-3-phenylprop-2-en-1-one (**3**), (*E*)-2-(1-hydroxy-3-phenylprop-2-en-1-ylidene)-4, 5-dimethoxy cyclopent-4-ene-1,3-dione(**4**) and 2-cinnamoyl-3-hydroxy-4,5-dimethoxycyclopenta-2, 4-dienone (**5**).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 IC_{50} values of 161.93 ± 0.004, 41.32 ± 0.005, 8.5 ± 0.004 , 157.58±0.002 and 149.45±0.01 ($\mu g/ml$), respectively, and FRAP with IC_{50}% values of 467 ±0.02 ,273.93±0.005 ,167.74±0.02 ,469.48±0.03 and 429.33±0.03. FIC very low

Keywords: Carbon dioxide, Ethanol, Water, Diffusion coefficients, Molecular dynamics.

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 flavonoids are much stronger than those of vitamins C and E [4].

We have performed a phytochemical study on the leaves and bark of a Malaysian Lauraceae, *Lindera Oxyphylla*, which has led to the isolation of 5- hydroxy-6,7-dimethoxy flavones(**1**) , 5,7 dihydroxy flavones(**2**), 2-propen-1-one,1-(2-hydroxy-4,5-dimethoxyphenyl)-3-phenyl(**3**),3-hydroxy-4,5-dimethoxy-2-[(2E)-1-oxo-3-phenyl-2-propen-1-yl](**4**) and4,5-dimethoxy-2-[(2E)-1-hydroxy-3-phynyl-2-propen-1,3-yl](**5).**

Result and Discussion

Biological Activity

Free radical scavenging activity (DPPH)

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.

*Corresponding author: Tel: +98 9111251481, E-mail: ma_hosseinzadeh@yahoo.com

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.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[6]. $DPPH^{\cdot}$ 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 $DPPH^{\cdot}$ 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 [8].

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

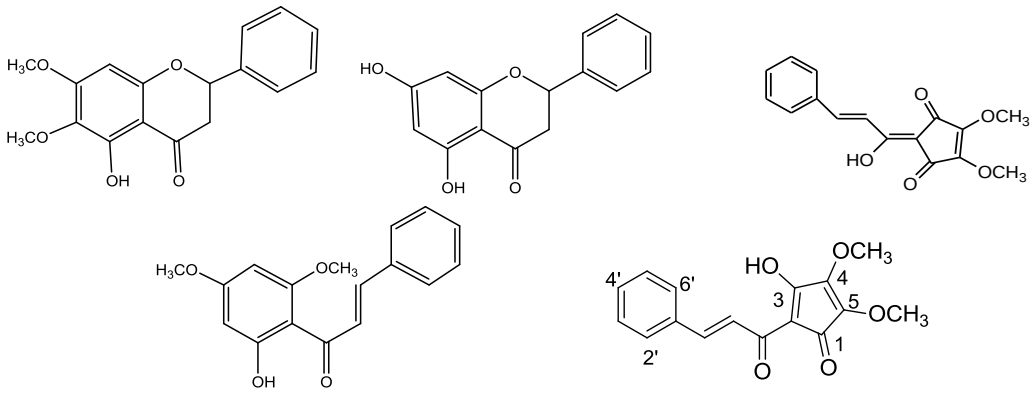

Figure 1: Structure of compounds **1-5**

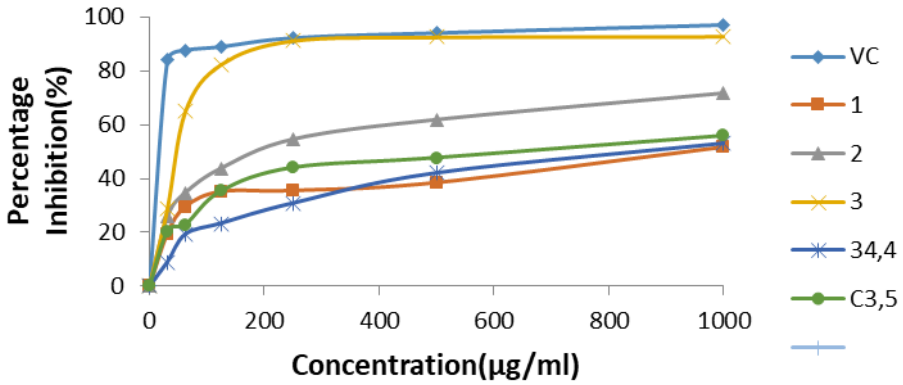

Figure 2: Scavenging activity of the isolated compounds of *Lindera Oxyphylla*

FRAP assay

The ferric reducing-antioxidant power was measured accordinto the method described by (8) with some modification. Generally induced by transition metal ions like Fe(III) in

thepresence 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.

$$Fe(III) + ArOH \rightarrow Fe(II) + ArO^{\cdot} + H+$$
$$ArO^{\cdot} + LH \rightarrow ArOH + L^{\cdot}$$
$$L^{\cdot} + O2 \rightarrow LOO^{\cdot}$$
$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot}$$
$$Fe(II) + LOOH \rightarrow Fe(III) + LO^{\cdot} + OH^{-}$$

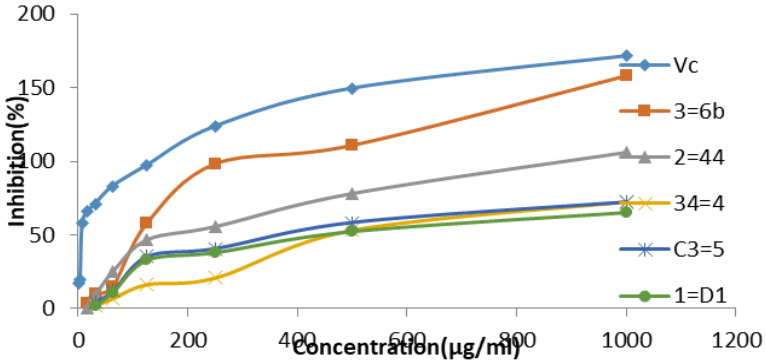

Figure 3: ferric reducing-antioxidant power of the isolated compounds of *Lindera Oxyphylla*

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].

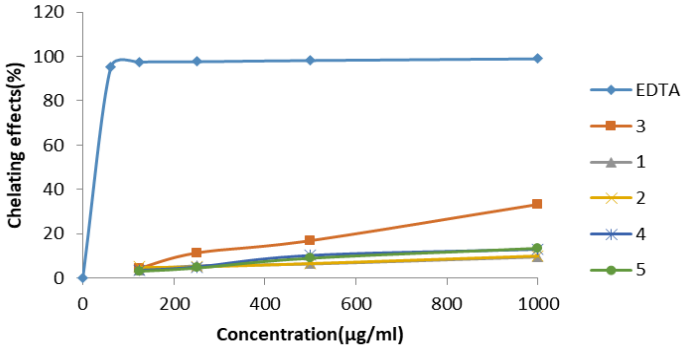

Figure 4: Cheleate effect of the isolated compounds of *Lindera Oxyphylla*

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 (Table **1**).

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
3	8.50 ± 0.004	107.74±0.04	Not found
4	57.58±0.02	469.48±0.03	Not found
5	149.45±0.01	429.33±0.03	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). The 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%.

Experimental Section

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 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 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 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 Flavonieds and Linderones

The dried bark (4 kg) of *Lindera Oxyphylla* were ground and extracted exhaustively with hexane (10.00 l) 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 Flavones 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). 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 (0.8 g). we can't sepreat alkaloids from Lindera.We repeat the extraction of Flavonoids by using MeOH solvent extraction obtained (20 g) crude Flavonoids. The crude 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; 450mL were used for each percentage) to yield 30 fractions. Fractions were then recombined on the basis of their TLC behavior to obtain eight fractions. Fractions 6-20 (?), afforded a Chalcone identified as *Lindera* (**3**) (%) (PTLC Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 98:2). Also, can be isolated from other fractions such as, fractions 22-30(4) (CH_2Cl_2-MeOH; 98:2) and fractions 34(5) crystal (CH_2Cl_2-MeOH; 99:1) by Methanol crude from leaf. (PTLC Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 98:2.

Determination DPPH radical scavenging activity

To 1.25 ml of 60 M DPPH in methanol, 250 L of each(31.25,62.5,125,250,1000 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]:

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

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 ferricyanide 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 trichloroacetic acid was added. The mixture was centrifuged at 3000 g for 10 min (Hermle Z300K centrifuge, Hermle Labortechnik GmbH, Wehingen Württ, Germany). The supernatant solution (0.5ml) was mixed with Water (0.5 ml) and 0.1 ml of 0.1% w/v FeCl3 to react for 10 min. Subsequently, the absorbance was measured at 700 nm. The EC50 value is the concentration of sample and the amount of gallic.

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

Antioxidant activity by Chelating effect on ferrous ions

The chelating of ferrous ions of compounds was estimated by the method of (?). Briefly, different concentrations of methanolic extract of compounds (125, 250, 500, 1000, g/mL) were added to a solution of 2mM FeCl2 (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:

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

where A_0 was the absorbance of the control and A_1 the absorbance in the presence of the sample of compound and standards (11).

Conclusion

Compound **3** 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.

Acknowledgements

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