Bioactive Compounds from the Leaves of *Lumnitzera racemosa* against Acetaminophen-induced Liver damage *in vitro*

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Abstract: Phytochemical investigation of the mangrove plant *Lumnitzera racemosa* WILLD (Fam: Combretaceae) has resulted in the isolation of 8 compounds by various chromatographic techniques (silica gel, ODS column chromatography and HPLC). The structures of these compounds were determined by spectrometric analysis (UV, IR, HR-ESI-MS, 1D and 2D-NMR). All the isolated compounds were evaluated for their hepatoprotective activity. The Compound number 8 showed high hepatoprotective activity against acetaminophen and compound 1 showed moderate activity compared to glycyrrhizin as the positive control using human Hep G2 cell line. The Compounds number 2, 3, 4, 5, 6 and 8 showed the highest DPPH radical scavenging activity (IC50: 14.74±5.66, 18.88±3.56, 5.93±2.1, 7.17±0.42, 7.38±1.03 and 15.72±1.18 μM, respectively), while compounds 1 and 7 showed lowest DPPH radical scavenging activity comparable with the standard trolox (IC50: 5.93±2.19 μM).

Key Words: Combretaceae; hepatoprotective, *Lumnitzera racemosa*, 1, 1-diphenyl-2-picrylhydrazyl (DPPH).

1. Introduction

Liver diseases are considered to be one of the most dangerous health problems around World. The incidence of liver cancer, including hepatocellular carcinoma, has been increased seriously in many countries (Ryder 2007). Major etiological factors contributing to hepatocellular carcinoma are well established and include viral infections, excessive ethanol consumption, environmental carcinogens, and hemochromatosis. Specifically, acetaminophen (APAP) is a well-known analgesic that is well known as a hepatotoxic agent in many European countries (Kaufman 2002, Blazer 2009, Wilcox 2005) and one of the most known drugs in the world. The data of the overdose of APAP showed its ability to increase the incidence of liver failure (Davidson 1966). Exposure of the liver to the free radicals derived from some xenobiotics and drugs leads to oxidative stress, which is recognized to be an important factor responsible for liver injury or be involved in the pathogenesis of liver disorders (Bandaranayake 2002, Ashok 2001). Therefore, studies on scavenging free radicals or reactive oxygen species (ROS) as well as reducing oxidative stress, and thereby avoiding hepatotoxicity, have received much attention. Biochemical studies suggested that toxic doses of APAP could cause changes in the morphology and function of liver mitochondrial (Placke 1987, Meyers 1988). APAP binds more frequently to mitochondrial proteins (Tirmenstein 1989), and causes mitochondrial oxidative stress (Jaeschke 1990). In the bioassay directed searching for hepatoprotective agents from the natural sources, employing the closely relevant model system to human liver toxicosis could be an effective way to identify therapeutically applicable agents, using most widely drugs in the world APAP. *Lumnitzera racemosa* Willd (Fam: Combretaceae) is a shrub or a small tree found on the coast of Japan, India and on the Andaman and Nicobar Islands. The reddish brown bark contains 15-19% tannins, while the leaves and wood contain smaller quantities. A fluid obtained from incisions made in the stem was reported to be useful for the treatment of herbs and itches (The Wealth of India 1962). Antihypertensive activity has been recently reported for the aqueous acetone extract of the plant (Lin 1993). Marine halophytes, such as mangroves and related species, are known to have many and various metabolites possessing antibacterial, antifungal (Abeysinghe 2006, Ravikumar 2009, Ravikumar 2010), antiviral (Zandi 2008), anti-diarrheal (Rouf 2007), hepatoprotective (Ravikumar 2011), anti- feedant (Wu 2008), insecticidal (Calderon 2001), cytotoxic (Han 2007), and antiplasmodial activities (Kim 1997). Chemical examination of this plant occurring in various parts of the world was reported to give a large number of compounds, long chain rubber like polysisoprenoid alcohols in leaves (Skoczylas 1994), flavonoids and long chain fatty acids and low molecular weight carbohydrates (Popp 1984). Chemical examination of the Indian species was reported to give friedelin, β-amin, taraxerol, betulin, β-sitosterol and triacantanol (Majumdar 1980). The presence of trace elements was also reported (Bhosale 1979). The present study deals with investigation of the chemical constituents of *L. racemosa* plant collected from marine regions in Japan, in addition to evaluation of
hepatoprotective and DPPH radical scavenging activities of the isolated compounds.

2. Materials and Methods

2.1. General experiments procedures

Optical rotation data were measured on a JASCO P-1030 Polarimeter. IR and UV spectra were performed using a Horiba FT-710 Fourier transform infrared and a JASCO V-520 UV/Vis spectrophotometers, respectively. 1H and 13C NMR spectra were recorded on a JEOL JNM α-400 spectrometer with tetramethyl silane as an internal standard. HR-ESI mass spectrum was taken on a LTQ Orbitrap XL mass spectrometer. Silica gel column chromatography (CC) was performed on Silica gel 60 (E. Merck, Darmstardt, Germany), 70-230 mesh. Reversed-phase [octadecysilanized silica gel (ODS)] open CC (RPCC) was performed on Cosmosil 75C18-OPN (Nacalai Tesque,Kyoto,Japan) (HPLC) was performed on an ODS column Inertsil ODS-3; GL Science,Tokyo,Japan (RPCC) was performed on Quercetin 3-O-(2''-O-galloyl)-α-rhamnopyranoside (U-E-13-5-3) and Myricetin 3-O-(2''-O-galloyl)-α-rhamnopyranoside (U-E-13-5-2).

3. Biological Assay

3.1. Cell culture

Human liver hepatoma cells (HepG2) (RIKEN Cell Bank: RCB1886), human cell line derived from hepatocyte carcinoma or hepatoblastoma of 15 years old male Caucasian, were cultured in RPMI1640 (R8758, SIGMA) containing 10%FCS, and antibiotics cocktail. Amphotericin B (SIGMA A9528 100 mg) and Kanamycin sulfate 5 g (WAKO 119-00703) are dissolved in 8.93 and 25 ml of sterilized MilliQ water, respectively. Equal volume of these solution, amphotericin B solution (8.93 ml) and kanamycin sulfate solution (8.93 ml) was combined, dispensed into microtube with 500 µl and stored at -20 °C.

MTT (3(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) (23547-21 1 g, Nacalai tesque) was dissolved in 181.8 ml of sterilized MilliQ water (5.5 mg/ml), and then dispensed into microtube with 1ml of solution and store at -20°C.

3.2. Hepatoprotective activity assay

After 3 days subculture, 100 µL of diluted cell suspension were added to the wells of 96-well microtitr plates (3×10^4 cells/ well), then cultured in a 5% CO₂ incubator at 37 °C for 24 h. The supernatant was aspirated off and then 100 µL of test compounds dissolved in DMSO in the presence of APAP (20 mM) were added to the each well of the 96-well microtitr plate at the final concentration of 1%, and then incubated at 37°C for 36 h. After that a MTT solution was added to each well and the plates were incubated for a further 1.5 h. Then the formazan precipitates were dissolved in 100 µL of DMSO. The absorbance was measured using a Molecular Device Versmax tunable microplate reader at 570 nm. Glycyrrhizin was used as a positive control.

%Viability

\[
= \frac{(A_{\text{sample}} - A_{\text{background}})}{(A_{\text{DMSO}} - A_{\text{background}})} \times 100
\]

The concentration of vehicle (DMSO) in each well is adjusted to contain 1%.
4. Results and Discussion

From the EtOAc fraction of a MeOH extract of the leaves of *L. racemosa*, eight compounds were isolated. One new compound (new cyclic compound) 1 and the structures of known compounds were determined to be 3,4-dihydroxy benzoic acid, 3 (Ashok 2001), 3,4,5-trihydroxybenzoic acid methyl ester 2 (Kai-Jin 2007), Loliolide 7 (Xiu-dong 2011), Quercetin-3-O-(2''-O-galloyl)-α-rhamnopyranoside 4, Myricetin 3-O-(2''-O-galloyl)-α- rhamnopyranoside 5, Sophoretin 6, Lyoniresinol 8 (Guangli 2012) (Fig. 1). The hepatoprotective activity and DPPH radical scavenging activity were evaluated in Figures 2-4.

![Chemical structure of Compound 3, 4-dihydroxybenzoic acid.](U-E-10-1-1)

![Chemical structure of Compound Benzoic acid. 3,4,5-trihydroxy-methyl ester](U-E-10-1-2)

![Chemical structure of compound][3]

![Chemical structure of compound][4]

![Chemical structure of compound][5]

![Chemical structure of compound][6]

![Chemical structure of compound][7]

![Chemical structure of compound][8]

Fig. 1. Structures of the isolated compounds 1-8.

![Hepatoprotective activity of *L. racemosa* EtOAc compounds using HepG2 cell line induced by APAP. Viability percentages are expressed as mean values± S.D. of 3 experiments.](Fig. 2)

![DPPH radical scavenging activity of isolated compounds. Inhibition percentages are expressed as mean values± S.D. of 3 experiments.](Fig. 3)

![IC50 of different compounds against DPPH free radical.](Fig. 4)
5. Conclusion

Overall, it could be concluded that *Lumnitzera racemosa* protects against oxidative injury induced by APAP in vitro using Hep G2 cells and the chemical constituents in this plant responsible for the hepatoprotection activity.

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References


