Separation of *Leucas aspera*, a Medicinal Plant of Bangladesh, Guided by Prostaglandin Inhibitory and Antioxidant Activities

Samir Kumar SADHU, Emi OKUYAMA,* Haruhiro FUJIMOTO, and Masami ISHIBASHI

Graduate School of Pharmaceutical Sciences, Chiba University; 1–33 Yayoicho, Inage-ku, Chiba 263–8522, Japan. Received December 24, 2002; accepted February 18, 2003

According to the traditional usage of the plant for antiinflammation and analgesia, *Leucas aspera* was tested for its prostaglandin (PG) inhibitory and antioxidant activities. The extract showed both activities, i.e., inhibition at 3×10^{-4} g/ml against PGE_{1}- and PGE_{2}-induced contractions in guinea pig ileum and a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect. The separation guided by the activities in these dual assay methods provided eight lignans and four flavonoids, LA-1—12, among which LA-1—7 and LA-10—12 were identified as nectandrin B, meso-dihydroplicarin A, macleignan, acacetin, apigenin 7-O-(6'-O-(p-coumaroyl))-\beta-D-glucoside, chrysoeriol, apigenin, *erythro*-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)propan-1-ol, and masticoside, respectively. LA-8 was determined to be (−)-chicanine, the new antipode of the (+) compound, by spectroscopic methods including CD and ORD. Chiral-HPLC analysis of LA-9 showed that it was a mixture of two enantiomers, (7R,8R)- and (7S,8S)-licarin A. All of these components were first isolated from *L. aspera*. PG inhibition was observed in LA-1, LA-2, and LA-5, and antioxidant activity in LA-1—3 and LA-8—12.

Key words  *Leucas aspera*; prostaglandin inhibition; antioxidant activity; lignan; (−)-chicanine

*Leucas aspera* Link (Labiatae) (darkolos or dandokolos in Bangladesh) is a common aromatic herb and grows abundantly in Bangladesh and also in the wide area of South Asia. Traditionally, the decoction of the whole plant is taken orally for analgesic-antipyretic, antirheumatic, antiinflammatory, and antibacterial treatment, etc., and its paste is applied topically to inflamed areas. Some reports have been published on the chemical constituents such as sterols, fatty acids, lactones, long-chain compounds, aliphatic ketols, and phenols. However, the biological activities of this plant have not been studied, except for antifungal effects. In our continuous research on traditional medicines concerning their herbal usage, the extract of *L. aspera* indicated prostaglandin (PG) inhibition in the Magnus method and antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The paper deals with the activity-oriented isolation of the extract using dual assay guides to identify the bioactive components of this plant.

The MeOH extract of *L. aspera* 3×10^{-4} g/ml showed inhibition against both PGE_{1}- and PGE_{2}-induced contractions in guinea pig ileum. It also showed positive (discolored) spots with a reddish purple background on TLC using DPPH as a spray reagent. After removal of chlorophylls by Diaion HP 20 column chromatography, the extract was evaluated for antioxidant activity (IC_{50} ca. 100 μg/ml) using a microplate reader with DPPH reagent. It was then partitioned with n-hexane, n-BuOH, and water, and the PG inhibitory activity was concentrated in the n-BuOH fraction. The DPPH radical scavenging effect was mostly observed in the n-hexane and n-BuOH fractions. Based on these results, the n-BuOH fraction was further separated by Sephadex LH-20 column chromatography to obtain the fractions with PG inhibitory activity. Fr. 1-B—1-E. Among them, fr. 1C and fr. 1D also showed clear DPPH-positive spots on TLC. Both fractions were then separated independently by repeated column chromatography by targeting DPPH-positive spots on TLC. Three major components, LA-1—3, together with five minor ones, LA-8—12, were obtained (Fig. 1). LA-1—3 and LA-10—12 were identified as nectandrin B, meso-dihydroplicarin A, macleignan, acacetin, apigenin 7-O-(6'-O-(p-coumaroyl))-\β-D-glucoside, chrysoeriol, apigenin, *erythro*-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)propan-1-ol, and masticoside, respectively.

* To whom correspondence should be addressed. e-mail: emi@p.chiba-u.ac.jp © 2003 Pharmaceutical Society of Japan
(5.2 μM) against PGE1-induced contraction, but was inactive against PGE2, at the same concentration.

In the case of antioxidant activity on TLC sprayed with DPPH reagent, all the lignans and neolignans LA-1—3 and LA-8—12 showed positive spots on TLC, whereas the flavonoids LA-4—7 were negative. The IC50 values of LA-1—3 and LA-5 and quercetin as a positive control recorded on a microplate reader with DPPH were 60, 28, 50, >500, and 30 μM, respectively. The antioxidant activity of nectandrin B (LA-1), meso-dihydroguaiaretic acid (LA-2), and macelignan (LA-3) have been already reported to have IC50 values of 74, 35, and 69 μM, respectively, using ESR spectroscopy.25 It was reported that meso-dihydroguaiaretic acid (LA-2) significantly preserved the levels and activities of glutathione, superoxide dismutase, glutathione oxidase, and catalase, and ameliorated lipid peroxidation.26 Acacetin (LA-4), chrysoeriol (LA-6), and apigenin (LA-7) were reported to have IC50 values of >500 μM as a result of DPPH assay.27

*L. aspera* has been used traditionally for its analgesic, anti-inflammatory, and antirheumatic properties. To find out the components responsible for the efficacy of this plant, we used dual assay methods, PG inhibitory and radical scavenging activities, since PGs and reactive oxygen and nitrogen species are closely related to inflammation and rheumatoid arthritis.30—34 From this point of view and our results, the major active components, LA-1—3 and LA-5, together with other minor ones, may contribute to the efficacy through inhibition of the inflammatory process. Some flavonoids, such as acacetin (LA-4), chrysoeriol (LA-6), and apigenin (LA-7), did not indicate any activity in this experiment. The following reports, however, suggested their contribution to the anti-inflammatory effect as well: acacetin for inhibition of COX and 5-LOX; apigenin for the inhibitory effect on NO production and PGE2 release; a reduction of iNOS and COX-2 expression; suppression of the LPS-induced activation of NF-kB; and an inhibitory effect on some other inflammatory mediators.30—34 In vivo effects of chrysoeriol and apigenin were reported using TPA-induced mouse ear edema and carrageenan-induced rat paw edema, respectively.33,34

Compounds LA-1—12 were first isolated from *L. aspera*. The separation of the remaining PG inhibitory fraction is continuing.
The UV detection wavelength was 254 nm. Following conditions: column, SHISEIDO CD-Ph (4.6 mm, 5 mg) together with additional crude methoxyphenoxypropan-1-ol. Colorless amorphous. Soluble part (187 mg) afforded (92 mg) was isolated by silica gel flash-column chromatography with n-hexane: acetone 1:1. The 5/1 eluate (22 mg) having PG inhibition yielded (43 mg). FAB-MS (NBA) \( m/z \) 243, \( t_{R} \) 0.59 (3H, d, \( J_{5,6} \) 9.4, CHCl₃), \( m/z \) 221 (nm), \( t_{R} \) 0.96, CHCl₃, \( m/z \) 239, \( t_{R} \) 1.04, CHCl₃. HPLC with MeOH:water 2/1 or Sephadex LH-20 with MeOH. Two major ones, \( R_{f} \) 0.5 and 0.1, inhibited both PGE₁- and PGE₂-induced contractions at concentration required to inhibit DPPH radical formation by 50%, calculated from the log-dose inhibition curve. Quercetin was used as a positive control at a concentration of 3 \( \times 10^{-6} \) M and 1 \( \times 10^{-5} \) M.

**Antioxidant Assay**

The antioxidant activity was evaluated based on the DPPH radical scavenging effect. The qualitative assay on TLC, activity was detected as in our previous reports, and ascorbic acid was used as a positive control. Spectrophotometric assay was performed by modification of the reported microplate method to determine \( IC_{50} \) values. To 10 \( \mu l \) of sample-DMSO solution in each microwell, 190 \( \mu l \) of DPPH-MeOH solution was added (final concentration of DPPH was 200 \( \mu M \)). After mixing in a microplate mixer for 30 min at room temperature, the absorbance was determined at 540 nm using a microplate reader (BIO-RAD Model 550). Each sample was measured in triplicate, and the mean result was taken. The antioxidant activity was expressed in terms of \( IC_{50} \) (\( \mu M \) and/or \( \mu g/Ml \), concentration required to inhibit DPPH radical formation by 50%), calculated from the log-dose inhibition curve. Quercetin was used as a positive control.

**Acknowledgments**

We thank the chemical analysis center of Chi Ba University for the measurement of FAB-MS.

**References**

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