Bioassay-guided fractionation of the methanol extract from the root of *Sophora flavescens* led to the isolation of eight known prenylated flavonoids responsible for the vasorelaxation activity in porcine coronary arteries. Among them, kushenol N and 5-methylsophorafavanone B strongly induced the relaxation of porcine coronary arteries with respective ED50 values of 8.6 and 12.4 μM. This activity and the results of a high-performance liquid chromatographic analysis suggest that kushenol N and 5-methylsophorafavanone B could be active markers in the *S. flavescens* extract for vasorelaxation activity.

**Key words:** vasorelaxation activity; *Sophora flavescens*; prenylated flavonoid; kushenol N; 5-methylsophorafavanone B

Cardiovascular diseases are the major cause of death in the developed countries. Dysfunction of the vascular endothelium is a hallmark of cardiovascular diseases and is characterized by multiple factors, including impaired vasodilation, tissue perfusion, hemostasis, and thrombosis.1-3 Substantial evidence suggests that plant-derived flavonoids are associated with decreased global mortality due to a reduced number of cardiovascular diseases and might be involved in protecting against cardiovascular risk.2,3

During our previous research work aimed at discovering new cardiovascular protective agents, about three hundred different medicinal plants used in oriental medicine were screened by evaluating their vasorelaxation activity of the solvent extract.4 Among them, the extract from the roots of *Sophora flavescens* Ait. (Leguminosae) was found to exhibit potent vasorelaxation activity in the porcine coronary artery rings. Bioassay-guided fractionation of this plant extract, using an assay that evaluated the relaxation of porcine coronary arteries preconstricted with the thromboxane mimetic, U46619 (1 to 60 nm) to about 80% of the maximal constriction, and the integrity of the endothelium was checked with bradykinin (0.3 μM). After washing out and a 30-min equilibration period, the rings were again constricted with U46619 before a concentration-relaxation curve was constructed for the total extract or each fraction. Each result is shown as the mean ± SEM of 4 to 6 different experiments. A statistical evaluation was performed with Student’s *t* test for paired data or by ANOVA with subsequent Fischer’s protected least significant difference test where appropriate. *p* and ***p*** respectively represent *p* < 0.05 and 0.01 compared with the control (0.1% DMSO).

Eight prenylated flavonoids in the bioactive methylene chloride fraction (2 g) were purified by semi-preparative HPLC with an acetonitrile-H2O gradient (35:65 to 70:30 in 60 min, 70:30 in 10 min, 10 mL/min, 210 nm).6 The HPLC chromatogram of *S. flavescens* (Fig. 2A) showed kurarinone (4) and sophorafavanone G (6) to be the major components of the *S. flavescens* extract. The chemical structures of these isolated compounds were elucidated to be 3,7,4-trihydroxy-5-methoxy-8-prenylflavanone (1, 24.8 mg),7,8 5-methylsophorafavanone B (2, 38.4 mg),7 kushenol N (3, 47.3 mg),8 kurarinone (4, 154.5 mg),8,9 2′-methoxykurarinone (5, 22.0 mg),9 sophorafavanone G (6, 104.0 mg),8,10 leachianone A (7, 31.6 mg),9,10 and kurarin (8, 34.7 mg)8 (Fig. 2) by

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2 Chul Young Kim and Hyun Jung Kim contributed equally to this study.
using NMR and MS spectroscopic methods and comparing with reported data.

The vasorelaxation effect of each prenylated flavonoid was measured by an assay to evaluate the relaxation of porcine coronary arteries as previously described. The vasorelaxation effect of each prenylated flavonoid was measured by an assay to evaluate the relaxation of porcine coronary arteries with EC50 values of 8.6 ± 4.1 and 12.4 ± 7.5 μM. Peaks: 1, 3,7,4'-trihydroxy-5-methoxy-8-prenylflavanone; 2, 5-methylsophorafavanone B; 3, kushenol N; 4, kurarinone; 5, 2'-methoxykurarinone; 6, sophorafavanone G; 7, leachianone A; 8, kurarinid; and EGCG, epigallocatechin gallate.

Fig. 2. HPLC Trace of the Active Methylene Chloride Fraction (A), and Chemical Structures and Vasorelaxation Activities (B) of Prenylated Flavonoids Isolated from *S. flavescens*. Kushenol N (3) and 5-methylsophorafavanone B (2) exhibited potent vasorelaxation effects on porcine coronary arteries with respective ED50 values of 8.6 ± 4.1 and 12.4 ± 7.5 μM. Peaks: 1, 3,7,4'-trihydroxy-5-methoxy-8-prenylflavanone; 2, 5-methylsophorafavanone B; 3, kushenol N; 4, kurarinone; 5, 2'-methoxykurarinone; 6, sophorafavanone G; 7, leachianone A; 8, kurarinid; and EGCG, epigallocatechin gallate.

All seven tested flavanones (1–7) with 8-prenyl or 8-lavandulyl substituents exhibited significant vasorelaxation activity in our study. It was not possible to derive accurate structure-activity information due to the limited set of 8-prenylated flavanones. However, activity data indicated that the vasorelaxation activity was associated with the presence of a substituent at C-3 and by its configuration (2 > 1; 3 > 4, Fig. 2B) in the 8-prenylated flavanones.

Kushenol N (3) and 5-methylsophorafavanone B (2) were also major compounds that exhibited stronger vasorelaxant activities than other compounds (Fig. 2). These two compounds could be marker for standardization of crude extract from roots of *S. flavescens* for vasorelaxation effect.

An *S. flavescens* extract has recently induced relaxation of phenylephrine-precontracted aortic rings, its vasorelaxation mechanism involving the release of nitric oxide from the endothelium, activation of soluble guanylyl cyclase-cGMP signaling in the vascular smooth muscle, and inhibition of Ca2+ entry via blockade of the L-type Ca2+ channels through cGMP signaling. Taken together, the present findings indicate that the vasorelaxation effect of the *S. flavescens* extract was mediated, at least in part, by 8-prenylated flavanoids. They further suggest that 8-prenylflavonoids would be a promising structural template for new cardiovascular protective agents of natural origin.

**Acknowledgments**

This work was supported, in part, by Research Funds of Mokpo National University in 2011.

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