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Research Note

Lipoxygenase Inhibitory Activity of Korean Indigenous Mushroom Extracts and Isolation of an Active Compound from *Phellinus baumii*

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Abstract We investigated a total of 335 samples of Korean native mushroom extracts as part of our lipoxygenase (LOX) inhibitor screening program. Among the mushroom-methanolic extracts we investigated, 35 exhibited an inhibitory activity greater than 30% against LOX at a concentration of 100 μg/mL. Especially, *Collybia maculata*, *Tylopilus neofelleus*, *Strobilomyces confusus*, *Phellinus gilvus*, *P. linteus*, *P. baumii*, and *Inonotus mikadoi* exhibited relatively potent LOX inhibitory activities of 73.3%, 51.6%, 52.4%, 66.7%, 59.5%, 100.0%, and 85.2%, respectively. Bioassay-guided fractionation led to the isolation of inoscavin A from the methanolic extract of *P. baumii*, which showed the most potent activity and was identified by spectroscopic methods. Specifically, inoscavin A exhibited potent LOX inhibitory activity with an IC_{50} value of 6.8 μM.

Keywords Inoscavin A, Korean native mushroom, Lipoxygenase inhibitor, *Phellinus baumii*

Mushrooms are classified as a fungus and consist of a spore-bearing fruiting body. Mushrooms are not only a nutritious food, but are also an important source of therapeutic medicines [1, 2]. Indeed, mushrooms produce various classes of secondary metabolites with diverse biological activities, including immunomodulatory, cardiovascular, anti-inflammatory, antidiabetic, antiviral, antioxidant, antitumor, and antimicrobial properties [2-4]. Due to these properties, mushrooms have been recognized as a source for the development of medicines and nutraceuticals [4].

The inflammatory response is initially regulated by the oxidation of polyunsaturated fatty acids, which catalyze three classes of enzymes including cyclooxygenase, cytochrome P450 and lipoxygenase. Lipoxygenases (LOXs) are a family of non-heme iron-containing enzymes that catalyze molecular oxygen into polyunsaturated fatty acids with a *cis*, *cis*-1,4-pentadiene structure, and are also involved in the first step of leukotrienes (LTs) formation from arachidonic acid via a radical mechanism. LOXs are widely found in plant, fungal, and animal species, and are classified as 5-, 8-, 9-, 12-, or 15-LOX on the basis of their site of substrate oxygenation [5]. It has been reported that LTs produced by the activities of LOXs play a major role in numerous inflammatory and immune responses related to several diseases including asthma, atherosclerosis, and cancer [6-8]. In addition, large quantities of LTs have been observed in pathophysiological conditions of asthma, psoriasis, rheumatoid arthritis, colitis ulcerosa, and allergic rhinitis. Therefore, inhibition of the biosynthesis of inflammatory mediators by blocking the activities of LOXs has been proposed as an effective therapeutic strategy for attenuating and protecting against inflammatory and allergic human diseases [9-11].

In our search for LOX inhibitors, we investigated 335 samples of indigenous mushrooms collected in Korea by soybean LOX enzyme assay. The collected mushroom samples were identified, cleaned to remove any residual compost/soil, and then ground and extracted with methanol. The resulting extract was then filtered to eliminate the sample residue, and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator. Next, the concentrates were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL, and their LOX inhibitory activities were estimated. We analyzed the extracts using the LOX enzyme assay as described previously, with

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minor modifications. Briefly, a 50 μL aliquot of soybean LOX (50 ng protein/mL) was pre-incubated with 20 μL of test sample in 50 mM Tris-HCI buffer (pH 7.6) containing DMSO (0.2% v/v) at room temperature for 5 min in a 96-well plate [10]. The reaction was initiated by the addition of 50 μL of linoleic acid (300 μM) and incubated for 30 min at room temperature. The absorbance was measured at 234 nm using a microplate reader, and 1-phenyl-3-pyrazolidinone (phenidone) was used as a positive control. The results of this assay indicated that 35 samples of the methanolic extracts tested exhibited an inhibition activity of above 30% against LOX at a concentration of 100 μg/mL as shown in Table 1. Four species of the Hymenochaetaceae family, one species of the Tricholomataceae family, and two species of the Boletaceae family exhibited potent inhibitory activities of more than 50% against LOX. Specifically, *Collybia maculata*, *Tylopilus neofelleus*, *Strobilomyces confusus*, *Phellinus gilvus*, *P. linteus*, *P. baumii*, and *Inonotus mikadoi* exhibited relatively potent LOX inhibitory activities of 73.3%, 51.6%, 52.4%, 66.7%, 59.5%, 100.0%, and 85.2%, respectively at a concentration of 100 μg/mL (Fig. 1). Based on these results, we determined that the methanolic extract of *P. baumii* exhibited the most potent LOX inhibitory activity. Therefore, we attempted to isolate and identify the compound from the methanolic extract of *P. baumii* responsible for the LOX inhibitory activity.

The methanolic extract of *P. baumii* was concentrated under reduced pressure, and the aqueous resultant was partitioned consecutively between hexane, chloroform, ethyl acetate, and butanol and water. LOX inhibitory activity was observed in the chloroform-soluble portion. After concentration of the chloroform-soluble portion under reduced pressure, the concentrate was subjected to silica gel column chromatography and eluted stepwise with chloroform : methanol (100 : 1~1 : 1, v/v). An active fraction was subjected to repeated chromatography with a silica gel

Table 1. Lipoxygenase inhibitory activities of methanolic extracts of Korean indigenous mushrooms

Class	Order	Family	Genus	Species	Inhibition (%)
Pezizomycetes	Pezizales	Pyromenataceae	*Aleuria*	*aurantia*	44.2
Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*longistriata*	40.0
		Tricholomataceae	*Collybia*	*maculata*	73.3
			Clitocybe	*gibba*	33.1
		Mycenaceae	*Xeromphalina*	*campanella*	31.0
		Strophariaceae	*Hypholoma*	*lateritium*	33.3
			Gymnopilus	*aeruginosus*	40.6
		Agaricaceae	*Crucibulum*	*laeve*	33.1
			Lycoperdon	*perlatum*	43.4
			Agaricus	*dulcidulus*	35.6
			Calvatia	*craniiformis*	37.4
	Boletales	Gomphidiaceae	*Chroogomphus*	*rutilus*	33.6
		Boletaceae	*Boletus*	*subvelutipes*	31.7
			Leccinellum	*griseum*	31.6
			Strobilomyces	*confusus*	52.4
			Tylopilus	*neofelleus*	51.6
		Suillaceae	*Suillus*	*americanus*	42.9
				viscidus	40.8
		Tapinellaceae	*Pseudomerulius*	*curtisii*	39.4
			Tapinella	*panuoides*	30.3
	Russulales	Russulaceae	*Lactarius*	*pterosporus*	35.3
				volemus	33.7
			Russula	*neoemetica*	34.1
		Stereaceae	*Stereum*	*ostrea*	42.3
	Polyporales	Polyporaceae	*Trametes*	*hirsuta*	31.9
			Daedaleopsis	*confragosa*	42.2
			Trametes	*orientalis*	33.4
			Roseofomes	*subflexibilis*	43.4
		Fomitopsidaceae	*Fomitopsis*	*pinicola*	33.1
				nigra	45.8
	Hymenochaetales	Hymenochaetaceae	*Phylloporia*	*ribis*	32.9
			Phellinus	*gilvus*	66.7
				linteus	59.5
				baumii	100.0
			Inonotus	*mikadoi*	85.2
	Thelephorales	Thelephoraceae	*Thelephora*	*palmata*	37.9
			Polyozellus	*multiplex*	36.4

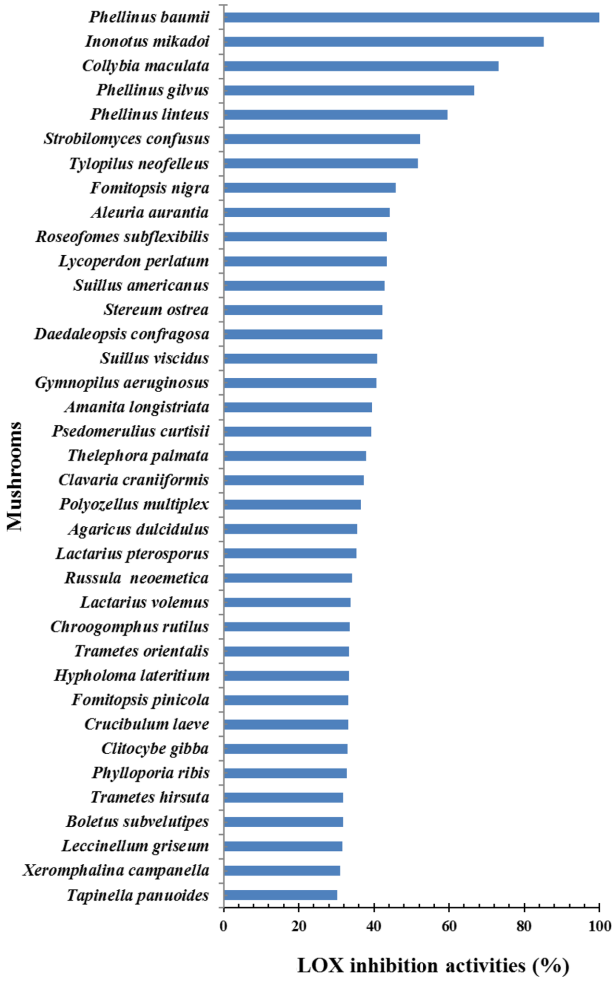

Fig. 1. Lipoxygenase inhibitory activities of methanolic extracts of Korean native mushrooms. LOX, soybean lipoxygenase.

column eluted with chloroform : methanol (5 : 1, v/v). The resulting active fractions were combined, subjected to a reversed-phase (C_{18}) Sep-pak cartridge, and eluted with 50% aqueous methanol. Finally, the active fraction was chromatographed on Sephadex LH-20 column (Pharmacia, Uppsala, Sweden) and eluted with methanol to give the active compound.

The chemical structure of the compound isolated was determined by spectroscopic methods and comparison of high-performance liquid chromatography (HPLC) retention time with authentic compound. Electrospray ionization-mass measurement established a molecular weight of 462. The ^{1}H NMR spectrum in DMSO exhibited signals due to four hydroxyl protons at 9.63, 9.19, 9.19, and 9.12 ppm, two 1,2,4-trisubstitutcd benzene rings at 7.07, 6.78, and 7.01 ppm and 6.72, 6.66, and 6.53 ppm, two olefinic methines attributable to a *trans*-1,2-disubstituted double bond at 7.32 and 6.80 ppm, three singlet methines at 6.71, 5.66, and 5.64 ppm, and a methyl at 1.92 ppm. The ^{13}C NMR spectrum revealed 25 carbon peaks including carbonyl and ester carbonyl carbons at 199.6 and 157.5 ppm, respectively,

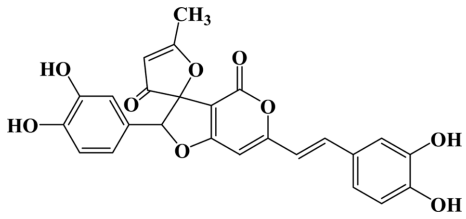

Fig. 2. Structure of the active compound, inoscavin A.

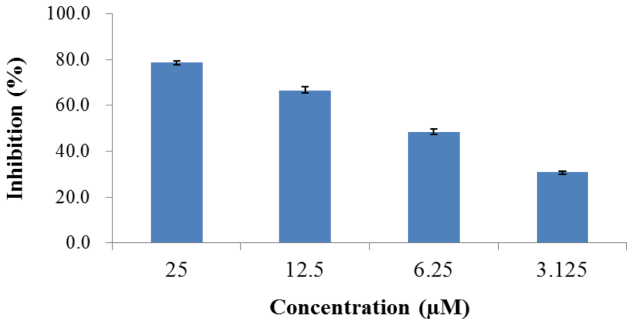

Fig. 3. Lipoxygenase inhibitory activity of the active compound, inoscavin A.

oxygenated sp^{2} quaternary carbons at 189.8, 174.2, 164.9, 148.2, 146.4, 145.7, and 144.9 ppm, as well as eleven methine carbons, four quaternary carbons, and a methyl carbon. The spectral data were in good agreement with those of inoscavin A, which was previously reported from the methanolic extract of *Inonotus xeranticus* [12]. Thus, we compared the HPLC retention time of the active compound isolated in this study with that of authentic inoscavin A. HPLC was performed with reversed-phase C_{18} column (150 × 4.6 mm i.d.) using a linear gradient starting at 10% aqueous methanol and reaching 100% methanol within a period of 30 min and acidified with 0.04% trifluoroacetic acid at a flow rate of 1 mL/min. Consequently, the HPLC retention time of active compound was the same as that of inoscavin A. Therefore, the active compound isolated was identified as inoscavin A (Fig. 2).

In conclusion, the LOX inhibitory activity of the isolated compound was estimated using soybean LOX. Inoscavin A exhibited potent LOX inhibitory activity in a dose-dependent manner, with an IC_{50} value of 6.8 μM (Fig. 3).

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