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Inhibitory Effects of the Stem Bark of *Albizia julibrissin* on Catecholamine Biosynthesis in PC12 Cells

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Abstract – The methanol extract of *Albizzia julibrissin* Durazz. (Leguminosae) was successively partitioned into dichloromethane, ethylacetate, butanol (BuOH) and water fractions, and the effects of the each solvent extract on catecholamine biosynthesis in PC12 cells were investigated. Among them, the BuOH fraction (5 μg/ml medium) showed 68.8% and 63.6% inhibition on dopamine and norepinephrine content in PC12 cells, respectively. Tyrosine hydroxylase (TH) activity was also reduced markedly by treatment of the BuOH fraction (41.8% inhibition at 5 μg/ml in the medium). Each solvent fraction did not show cytotoxicity towards PC12 cells by trypan blue exclusion test. This result suggests that the BuOH fraction has an inhibitory effect on catecholamine biosynthesis by reducing TH activity in PC12 cells.

Key words – *Albizia julibrissin*; Leguminosae; catecholamine biosynthesis; tyrosine hydroxylase; PC12 cells.

The stem bark of *Albizia julibrissin* Durazz. (Albiziae Cortex, Leguminosae) is a well known herbal medicine used as tonics, to ease the mind and calm the nerves. This drug shows a positive reaction of saponines which are identified for triterpene types.[1] Among the sapogenins of triterpene type, machaericnic acid methyl ester, acacic acid lactone, acacigenin B, machaericnic acid lactone and 16-deoxyacacigenin B were isolated.[2-4] The PC12 cells derived from rat adrenal pheochromocytoma exhibit many properties of the adrenal medullary chromaffin cells, including the synthesis, storage and secretion of catecholamines.[5,6] The PC12 cells also express tyrosine hydroxylase (TH), the rate-limiting enzyme of the catecholamine biosynthetic pathway.[5] In this study, the several fractions were partitioned from the methanol (MeOH) extract of *A. julibrissin*, and the effects of dichloromethane (CH_2Cl_2), ethylacetate (EtOAc), butanol (BuOH) and water fractions on catecholamine content and TH activity in PC12 cells were investigated.

The dried stem bark of *A. julibrissin* Durazz. was purchased from Han-Kook Sin Yak Pharm. Co. Ltd. (Taejeon, Korea). A voucher specimen is deposited in the Department of Pharmacology at College of Pharmacy, Chungbuk National University. All chemicals were of reagent grade. MeOH extract (28.0 g), obtained from the dried stem bark of *A. julibrissin* (200 g), was suspended in water (1.0 L) and partitioned

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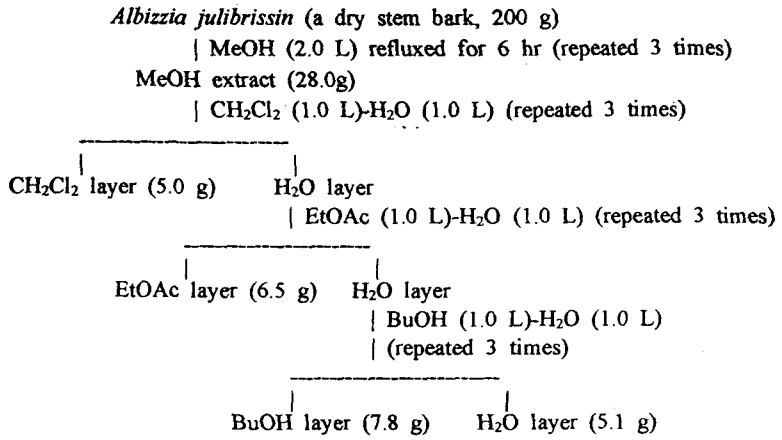

Fig. 1. Isolation of the active fractions from the stem bark of *Albizia julibrissin*.

three times with CH_2Cl_2 (1.0 L) and EtOAc (1.0 L) successively. The aqueous layer was extracted three times with BuOH (1.0 L). As the solvent was removed under reduced pressure, each extract and the aqueous layer were frozen and dried to a powder (CH_2Cl_2 fraction, 5.0 g; EtOAc fraction, 6.5 g; BuOH fraction, 7.8 g; H_2O fraction, 5.1 g) (Fig. 1). The PC12 cells were grown routinely as described.[6] The cells (ca. 1×10^5 cells/cm^2) were treated with each extract (5 μg/ml medium) and then incubated for 48 hr. The cells (ca. $1.5\text{-}2\times10^5$ cells/cm^2) were harvested and centrifuged. The pellet extract was used for the measurement of catecholamine content and TH activity. Catecholamine content was determined as described previously.[7,8] The TH activity was measured by a modification of the method of Nagatsu *et al.*[9] The conditions of the fluorescence derivatization for catecholamines and HPLC analysis were the same as described previously.[8] The amount of protein was determined by the method of Lowry *et al.*[10]

It has been previously investigated that the MeOH extract of *A. julibrissin* (5 μg/ml medium) shows an inhibitory effect on dopamine biosynthesis in PC12 cells (unpublished data). Therefore, the MeOH extract was successively partitioned into CH_2Cl_2, EtOAc, BuOH and H_2O fractions (Fig. 1). Among them, the BuOH fraction (5 μg/ml medium) showed 68.8% and 63.6% inhibition on dopamine and norepinephrine content in PC12 cells, respectively (Table 1). The EtOAc fraction also exhibited an inhibitory effect on dopamine content, but this was not significant. The secretion of catecholamines (dopamine and norepinephrine) into the medium slightly increased upon addition of the BuOH fraction (5 μg/ml medium), but these were not significant (data not shown). The cell viability was examined by trypan blue exclusion test. Each solvent fraction using as indicated concentration did not show cytotoxicity towards PC12 cells. Therefore, the catecholamine content, stored in the cells and secreted into the medium, was significantly reduced by the BuOH fraction in PC12 cells. TH activity was markedly reduced by treatment of the BuOH fraction (41.8% inhibition at 5 μg/ml in the medium) (Table 1). This result suggests

Table I. Inhibitory effects of the stem bark of *Albizia julibrissin* on the content of intracellular catecholamines in PC12 cells

Herbal medicine (5 μg/ml medium)	Catecholamine content (% of control)		TH activity (% of control) (nmol/min/mg protein)
	Norepinephrine (pmol/mg protein)	Dopamine (nmol/mg protein)	
Control	87.1±5.1 (100)	4.42±0.51 (100)	94.6±9.8 (100)
A. julibrissin			
CH_2Cl_2 Fr.	90.6±11.8 (104)	3.72±0.56 (84.2)	
EtOAc Fr.	68.1±12.6 (78.2)	3.30±0.35 (74.7)	
BuOH Fr.	31.7±6.8 (36.4)**	1.38±0.42 (31.2)**	55.1±6.7 (58.2)*
H_2O Fr.	71.2±15.5 (81.7)	4.22±0.43 (95.5)	

Cells were incubated for 24 hr and replaced by fresh media. The cells were treated with various fractions of *A. julibrissin* (5 μg/ml medium) and then incubated for 48 hr. The cells were harvested with PBS, and the catecholamine content and TH activity were measured by HPLC. Results represent the mean±SE of six dishes. Significantly different from the control value: *, $p<0.05$; **, $p<0.01$ (Student's t test).

that the BuOH fraction has an inhibitory effect on catecholamine biosynthesis by reducing TH activity in PC12 cells. TH activity in PC12 cells is responsible for various factors such as c-AMP, dexamethasone, growth factors, protein kinase A, and protein kinase C.[11),12)]

The stem bark of *A. julibrissin* is known to contain saponins of triterpene type.[2)-4)] Quercetin-3-O-galactoside, quercetin-3-O-rhamnoside,[13)] α-spinastenyl glucoside, 3′, 4′,7-trihydroxyflavone,[14)] syringaresinol glycosides[15)] and triterpenoidal glycosides[16)] were also identified. In various components of *A. julibrissin*, machaericnic acid methyl ester and acacic acid lactone obtained from saponin fraction have strong uterotonic acid.[2)] In addition, julibrissin II, one of pyridoxine derivatives, obtained from the MeOH extract shows to exhibit arrhythmic-inducing action, heart toxicity.[17)]

The sapogenin derivatives such as machaericnic acid methyl ester and acacic acid lactone have been isolated from BuOH fraction of *A. julibrissin*.[2)-4)] Therefore, It has been hypothesized that the saponines from *A. julibrissin* might have an inhibitory effect on catecholamine biosynthesis in PC12 cells. BuOH fraction (40 μg/ml medium) from *Polygala tenuifolia*, which contains several Onjisaponin derivatives, also inhibited the catecholmaine biosynthesis in PC12 cells,[18)] but ginseng total saponins (40-80 μg/ml medium) did not show an inhibitory effect (data not shown).

In this experiment, BuOH fraction from *A. julibrissin* decreased the catecholamine content in PC12 cells. TH activity was also reduced by the BuOH fraction. The separation of bioactive component(s) from *A. julibrissin* as well as their mechanisms in PC12 cells need further investigation.

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