論　文　内　容　要　旨

Phytochemical study of Indonesian plants and microbial transformation

（インドネシア産植物の含有成分の分析と微生物変換研究）

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Abstract

Introduction

Natural products from medicinal plants provided opportunities for new drug discovery so far. Due to an increasing demand for chemical diversity, seeking therapeutic drugs from natural products has grown throughout the world. More than 80% of the medicinal plants in Southeast Asia can be found in Indonesia. The richness of diversity of Indonesian plants has been recognized throughout the world. However, the chemical constituents and biological activity of these plants have not yet been fully investigated. *Cleome rutidosperma* (Cleomaceae) is native to West Africa and has naturalized in Southeast Asia, including Indonesia, and used traditionally as a stimulant, antiscorbutic, anthelmintic, rubefacient, vesicant, and carminative. *Excoecaria agallocha* (Euphorbiaceae) is a typical mangrove species widely distributed in the wetlands in temperate and tropical regions of Asia, Africa, and Northwest Australia. Its extracts are known to have anti-diabetic, anti-tumor, and anti-bacterial properties as well as high antioxidant activity. *Chromolaena odorata* (Asteraceae) has many ethnopharmacological uses, including the treatment of malaria, wounds, diarrhea, skin infection, toothache, dysentery, stomachache, and colds. Different growing environments affect the production of secondary metabolites. Therefore, we investigated the chemical constituents of these three plants collected in Makassar, Sulawesi, Indonesia, and identified three (**1-3**), four (**4-7**), and one (**8**) compounds, respectively, from these plants.

Through our phytochemical research mentioned above, we have noticed the NMR assignment of morin-3-*O*-glucoside (**9**) should be re-investigated. Therefore, we prepared morin-3-*O*-glucoside (**9**) by microbial transformation of morin with *Cunninghamella* sp., and the NMR assignment was re-examined. The microbial culture also produced another compound (**11**). The NMR and MS analyses of **11** revealed it as a previously undescribed glucoside, morin-2'-*O*-glucoside (**11**). In this study, the identification of chemical constituents in three Indonesian plants, the revision of the NMR assignment of morin-3-*O*-glucoside (**9**), and the preparation and structural elucidation of morin-2'-*O*-glucoside (**11**) were described.

Materials and Methods

The leaves of *E. agallocha*, and aerial parts of *C. rutidosperma*, and *C. odorata* were collected in Makassar, Sulawesi, Indonesia. The air-dried materials were extracted with methanol and then partitioned with solvents of different polarity successively. The obtained fractions were separated by various chromatographic techniques, including HPLC. The chemical structures of the isolated compounds were determined by spectroscopic analyses.

C. echinulate (JCM22488) and *C. elegans* (JCM22489) were obtained from the Japan

Collection of Microorganisms (JCM) in RIKEN-BRC, Japan. The microbial transformation was performed according to the reported method. In brief, a modified YPD medium (D-glucose 5.0 g, Kyokuto peptone 0.5 g, and Yeast extract 0.3 g, in 100 ml of distilled water) in a 300 ml Erlenmeyer flask was sterilized by autoclave. A small piece (approx. 5 x 5 mm) of seed culture on a PDA plate was inoculated in the liquid media and cultured at 28 °C, 120 rpm for three days to achieve sufficient growth of mycelia. Then, 50 mg of morin dissolved in 500 µl of DMSO was added and cultured for up to eight days at the same condition. The efficiency of microbial transformation was monitored by TLC analysis. The day eight culture was extracted with EtOAc and purified by preparative TLC and HPLC to afford compounds **9** and **11**. The revision of the NMR assignment of **9**, and the structural elucidation of a novel compound (**11**) were performed using the spectroscopic method.

Results and Discussions

--- Phytochemical investigation of Indonesian plants ---

Intensive fractionation and isolation study resulted in the identification of three compounds (**1-3**) from *C. rutidosperma*, four compounds (**4-7**) from *E. agallocha*, and one compound (**8**) from *C. odorata*. The chemical structures were determined by various spectroscopic analyses. Of these, Compounds **1** and **2** were evaluated in DPPH radical scavenging assay, anti-*Leishmania*, and cytotoxicity against human lung cancer cells A549. No cytotoxicity was observed against *Leishmania* and A549, but both **1** and **2** showed significant activity in the DPPH radical scavenging assay.

--- Microbial transformation study of morin ---

Two strains, *C. echinulate* (JCM22488) and *C. elegans* (JCM22489), were used for microbial transformation. After sufficient growth of mycelium, an aliquot of morin solution in DMSO was added to the culture and monitored for up to eight days for the production of glucoside by TLC analysis. The substrate, morin (R_f = 0.55), completely disappeared in 4 days, and a more polar single spot appeared at R_f = 0.45. Both strains showed similar results, but the intensity of the spot from *C. echinulate* (JCM22488) was higher than that of *C. elegans* (JCM22489). This single spot was purified by preparative TLC, but the NMR analysis revealed the presence of two compounds. The mixture was then purified by preparative HPLC to yield compounds **9** and **11** at t_R = 11.0 and 15.5 min, respectively. Because no reliable NMR assignment of morin-3-*O*-glucoside has been reported so far, the structural elucidation was carefully performed by the analyses of 1D and 2D NMR and HR-ESI-MS. As a result, the structure of **9** was determined to be morin-3-*O*-*β*-D-glucopyranoside, and the previous NMR assignment was revised to be of quercetin-3-*O*-*β*-D-glucopyranoside. In the same way, compound **11** was determined as morin-2'-*O*-*β*-D-glucopyranoside as a novel compound.