

学 位 論 文 の 要 旨

論文題目 Study on plant-based natural products for drug discovery: ethnopharmacology to phytochemistry

(創薬を目指した植物由来の天然物の研究：民族薬理学から植物化学まで)

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Plants are a source of a wide range of natural products possessing various therapeutic properties and are constantly explored for developing new drugs. Now-a-day, numerous natural products obtained from medicinal plants are used in modern therapeutic systems and play a vital role in the development of drug discovery. Natural product research has origins in ethnopharmacology and has evolved through phytochemistry.

The present study investigated potential plant-based natural products in the light of ethnopharmacology and phytochemistry for future drug discovery. My doctoral dissertation comprises three parts focusing on exploring plant-based natural products. In the first part, I investigated and documented the therapeutic uses of medicinal plants from the world's largest mangrove forest Sundarbans from Bangladesh. The second part included isolating and characterizing novel melanin-like pigment from cultured tobacco BY-2 cells. Furthermore, in the third part, I evaluate the ultraviolet (UV) protection and antioxidant ability of the isolated tobacco BY2-melanin.

1. Exploring the therapeutic uses of plants employed by 'Munda' ethnic community lives around Sundarban mangrove forest

The Munda peoples are a small ethnic group that has lived in the vicinity of Sundarbans, the world's largest mangrove forest in the southwestern part of Bangladesh, for over two centuries. In the study, repeated field surveys were conducted, and interviewed among 79 Munda informants were to collect ethnopharmacological data. Informants were selected through random sampling techniques and interviewed using an open and semi-structured questionnaire. The collected data were analyzed using qualitative and quantitative approaches. I explored and compiled 3199 medicinal use reports (URs) for 98 medicinal plant species to treat 132 ailment conditions under sixteen pathological groups. The highest use reports (948) were noted for the digestive group treated by 69 plant species, where the highest informant consensus factor (ICF) value was measured for the social problem disease category (ICF: 1.00). Of the recorded medicinal plants, 17 were identified as true Mangrove (MNG), 24 as Mangrove Associates (MNA), and 57 as Non-Mangrove (NMG) species. Fabaceae (13 species) represented the leading family, followed by Lamiaceae and Compositae (5 species). Herbs (43%) have shown dominant life forms, and the leaves (41%) were frequently used plant parts. The most commonly cited preparation method was juice (24%), and the prevalent mode of administration was oral (62%). *Azadirachta indica* A. Juss. was the most widely used therapeutic plant species based on FC (39) values. The comparative literature review study reveals that the practices of 15 plants and their

ethnomedicinal use by the Munda people are still entirely unexplored and newly reported in Bangladesh. Additionally, therapeutic use of 2 species, *Brownlowia tersa* (L.) Kosterm., and *Dalbergia candenatensis* (Dennst.) Prain has not been previously reported worldwide. In addition, 51 plant species (52%) of the total plants studied have enlisted on the IUCN Red List of Threatened Species.

2. Isolation and characterization of a novel melanin-like pigment from cultured tobacco BY-2 cells

The tobacco (*Nicotiana tabacum*) cultured cell line BY-2 (referred to as "BY-2 cells") is one of the most utilized plant cell lines. In a typical BY-2 cell subculture in work, 3 mL of cultured cells in 75 mL of LSD medium in a 300 mL flask with shaking was repeatedly transferred to 75 mL of fresh LSD medium in a 300 mL flask every week. Such cultured cells did not turn brown in two weeks but were mostly brown three weeks after transfer. On the other hand, when 1 mL of cells cultured for a week was transferred to 20 mL of LSD medium in a 100 mL flask and cultured with shaking, the cells began to turn brown at around ten days and completely turned blackish-brown three weeks after transfer. The medium also turned blackish-brown, suggesting that some blackish-brown pigment was produced or accumulated inside the cells and secreted or leaked out from the cells. Therefore, to investigate whether melanin-like pigments accumulated in BY-2 cells, the isolation of a blackish-brown pigment from BY-2 cells has been accomplished, and analyzed its physicochemical properties. The blackish-brown particles were isolated from the cultured BY-2 cells by elution with 0.5 M NaOH and precipitation with HCl, followed by treatment with organic (ethyl alcohol, ethyl acetate, and acetone) solvents. More than ten independent extractions routinely yielded 150–170 mg of blackish-brown pigment from 5 g of dried BY-2 cells. The UV-Vis spectrum, Fourier transform infrared spectrometry, and Electron spin resonance (ESR) spectroscopy analyses of blackish-brown pigment were done and compared with synthetic and Sepia melanins. All the results revealed that the characteristics of this pigment possessed most of the properties of melanin-like pigments. Furthermore, as physicochemical properties, the pigment was insoluble in water and organic solvents, modestly soluble in low concentrations of HCl, well soluble in alkaline solutions, and soluble in some neutral to weakly alkaline buffer solutions. It was stable to heat, solar light, and metal ions while fading by UV and oxidants. The morphology of the isolated BY2 pigment was observed under optical microscopy and scanning electron microscopy (SEM) with the comparison of Sepia melanin. Morphologically, BY2 pigment was not in the form of black spherical particles like Sepia melanin but was dark brown amorphous particles of varying size. SEM observations revealed that the BY2-pigment particles were angular and irregular, with large particles of approximately 10 μm and small particles of about 2 μm . Hereafter, the isolated blackish-brown pigment is termed "BY2-melanin." The elemental composition of BY2-melanin contained C, H, and N, but not S, suggesting that BY2-melanin was different from pheomelanin, which contains S. Since the N content of BY2-melanin was about 10%, this melanin was different from allomelanin reported as plant melanin, suggesting the possibility of novel eumelanin-like pigment: BY2-melanin.

3. Evaluation of ultraviolet (UV) protection ability and antioxidant effects of BY2-melanin

In this study, the UV protection ability was tested by measuring the survival rate of the *Escherichia coli* DH5 α strain irradiated with UVA (360 nm), UVB (306 nm), or UVC (253 nm). The study's results showed that the survival rate of *E. coli* decreased slightly with UVA irradiation for 20 minutes and decreased by more than 90% with UVB or UVC irradiation for 0.5 minutes. In contrast, when I added BY2-melanin at a concentration of 2 mg/mL to the growth medium of *E. coli* and irradiated for 1 min, the survival rate of *E. coli* recovered to 90% or more at any UV irradiation. Antioxidant effects of the BY2-melanin were examined by its ability to scavenge DPPH radicals and superoxide radicals. The results showed that BY2-melanin had free radical scavenging

ability for both radicals, although BY2-melanin required a higher concentration than the other standard antioxidant and melanin-like substances. The UV protection and antioxidant properties of natural BY2- melanin will accelerate its potentiality in future industrial applications, particularly in the cosmetic and pharmaceutical industries.

Articles

[1]. Islam, ATM Rafiqul, Md Mahadiy Hasan, Md Tahidul Islam, and Nobukazu Tanaka. "Ethnobotanical study of plants used by the Munda ethnic group living around the Sundarbans, the world's largest mangrove forest in southwestern Bangladesh." *Journal of Ethnopharmacology* 285 (2022): 114853.

[2] Islam, A. T. M., Keita Shinzato, Hiroki Miyaoka, Kenji Komaguchi, Kanae Koike, Kenji Arakawa, Kenji Kitamura, and Nobukazu Tanaka. "Isolation and characterization of blackish-brown BY2-melanin accumulated in cultured tobacco BY-2 cells." *Bioscience, Biotechnology, and Biochemistry* (2023) (Accepted).