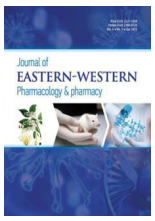

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KEY COMPOUNDS IN PLANTS EATEN BY MONGOLIAN LIVESTOCK DURING WINTER, *AMYGDALUS* PEDUNCULATA AS AN EXAMPLE

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KEYWORDS

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ABSTRACT

A wide varieties of plants are distributed across Mongolia, many of which serve as valuable medicinal and forage reserves for livestock in nomadic cultures. Their usage and characteristic features were investigated through the Science and Technology Research Partnership for Sustainable Development (SATREPS) program, conducted from 2020 to 2025. As part of this program, our group focused on studying and reporting the chemical constituents of these plants and their biological activities to understand the scientific basis underlying their traditional usages. A presentation at the conference, an overview of the SATREPS project, and its results will be presented. Among the various studies conducted, chemical constituents isolated from *Amygdalus pedunculata*, a valuable forage shrub, have been highlighted. The extract of *A. pedunculata* (a cyanogenic glycoside), namely prunasin, and four flavanones namely eriodictyol, naringenin, taxifolin, and aromadendrin were isolated as main constituents of the branches of *A. pedunculata*. These compounds may bring significance and effects on animals eating *A. pedunculata*.

INTRODUCTION

Many plant resources in Mongolia exhibit highly tolerance to cold and salinity and/or possess valuable medicinal properties. To restore pastures and improve the health of nomadic livestock, our group joined the Science and Technology Research Partnership for Sustainable Development (SATREPS) Program, conducted from 2020 to 2025. SATREPS1906 (Title: Restoration of Pastureland by Effective Usage of

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Wild Forage Plants based on Traditional Knowledge of Nomadic Mongolians; Principal Investigator: Prof. ASAMI Tadao, University of Tokyo; Javzan BATKHUU, National University of Mongolia) is a JST and JICA program for research projects targeting global issues and involving partnerships between researchers in Mongolia and Japan.[1)]

As one of the objectives of this project, studies have been conducted on plants distributed across Mongolia that appear to be related to nomads and livestock. Some of these plants are used as crude drugs (medicinal), while some are toxic but, have potential for use as forage plants.

Important plants for the Mongolian people and their livestock have been field-surveyed, and the project members have tried to evaluate the chemical constituents and biological activities of selected plants to better understand their characteristics and basis for their effectiveness.

Amygdalus pedunculata (= *A. mongholicus*; accepted name: *Prunus pedunculata* (Pall.) Maxim.) is a Rosaceae shrub distributed across the rocky and stony slopes in North-Central China, Inner Mongolia, Siberia Buryatiya, and Mongolia.[2)] Generally, the seeds of rosaceous plants are used for medicinal purposes. Furthermore, this species has also been analyzed for its fatty acid and sugar content in its seeds, and it is traditionally used as a laxative and diuretic.[3)] In addition to its high palatability for sheep and goats in spring and summer,[4)] it also serve as an important forage plant during the winter months. When no other wild forage plants are found, livestock feed on the young denuded branches of this shrub that are out of the snow. This suggests that this shrub is a valuable feed for animals to survive the harsh Mongolian winters. Hence the effects of its components on animals should be investigated.

In this conference, we present an overview of the SATPREPS Project and its results. Next, as an example, we report the results of an investigation of the chemical components of *A. pedunculata*.

MATERIALS AND METHODS

General: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), ^{1}H-^{1}H COSY, HMQC (optimized for $^{1}J_{C\text{-}H}$ = 145 Hz), HMBC (optimized for $^{n}J_{C\text{-}H}$ = 8 Hz), and NOESY spectra were recorded on a JNM-AL400 or JNM-ECZ400S/L1 FT-NMR spectrometer (Jeol Ltd., Tokyo, Japan) with chemical shifts expressed in ppm (δ) with respect to TMS as the internal standard. Methanol-d_4 and DMSO-d_6 were used as NMR solvents. FABMS data were obtained using a JMS700 mass spectrometer (Jeol Ltd.) with either *m*-nitrobenzyl alcohol or a glycerol matrix. A porous polymer gel (Diaion HP-20; Mitsubishi Chemical Co., Tokyo, Japan) was used for column chromatography. Preparative HPLC was performed on a Jasco 2089 instrument fitted with a UV detector (210 nm) [columns: ODS-SM-50C-M (Yamazen Co., Osaka, Japan, 37 × 300 mm), TSKgel ODS-80Ts (Tosoh, Tokyo, Japan, 21.5 × 300 mm), and Develosil C30-UG-5 (Nomura Chemical, Aichi, Japan, 20 × 250 mm)].

Plant material: The branches of *Amygdalus pedunculata* were collected from Arkhust soum (47°35′24.4″N, 107°58′01.3″ E; at an altitude of 1454 m), Tuv Province, Mongolia, on 28th October 2023. Professor Shagdar Dariimaa of the Mongolian State University of Education identified the plant species. A voucher specimen (No. 20231028) has been deposited at the Laboratory of Bioorganic Chemistry and Pharmacognosy at the National University of Mongolia.

Extraction and isolation: Young branches of *Amygdalus pedunculata* collected after leaf fall in autumn (306 g) were extracted using a acetone-water (4:1) mixture and concentrated to yield a crude extract (61 g). This extract (56 g) was subjected to column chromatography (Mitsubishi Diaion HP-20, 500 mL) to yield following fractions: Fraction 1A [H_2O, 9.5 g]; Fraction 1B [MeOH:H_2O (1:4, v/v), 0.3 g]; Fraction 1C [MeOH:H_2O (2:3, v/v), 10.2 g]; Fraction 1D [MeOH:H_2O (3:2, v/v), 5.9 g]; Fraction 1E [MeOH:H_2O (4:1, v/v), 1.4 g]; Fraction 1F [MeOH, 0.4 g]; Fraction 1G (acetone, 0.2 g). Reversed-phase HPLC separation and purification of fraction 1C yielded compounds **1** (152.6 mg) and **4** (40.6 mg); fraction 1D yielded compounds **2** (16.8 mg) and **4** (262.6 mg); and fraction 1E yielded compounds **2** (143.7 mg), **3** (190.1 mg), **4** (37.2 mg), and **5** (32.1 mg).

RESULTS and DISCUSSION

The isolated compounds were identified by comparing the spectroscopic data with existing references for prunasin (**1**),[5)] eriodictyol (**2**),[6)] naringenin (**3**),[7)] taxifolin (**4**),[8)] aromadendrin (**5**).[9)] The chemical structures obtained based on the ^{1}H and ^{13}C NMR spectra of **1** are shown in Figure 1. The assignments of the data were confirmed using 2D data, including ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY spectra (Figure 1). The spectroscopic feature of cyanogenic glycosides included the nitrile (δ_C 119.4) and benzyl (δ_C 68.4) carbons. Amygdalin, a cyanide glycoside that has attracted attention as a medicinal constituent of Rosaceae seeds, was not detected in this experiment. However, because prunasin has only one glucose molecule less than amygdalin, the two compounds have common biological activities. It is

the next issue what causes the difference in the content ratios between the two. Because hydrogen cyanide produced by the hydrolysis of prunasin and amygdalin is highly toxic to animals,[10] the effects of ingesting prunasin-containing plants need to be investigated in future studies. Based on these results and discussions, detailed quantitative analyses of the cyanide glycosides were planned.

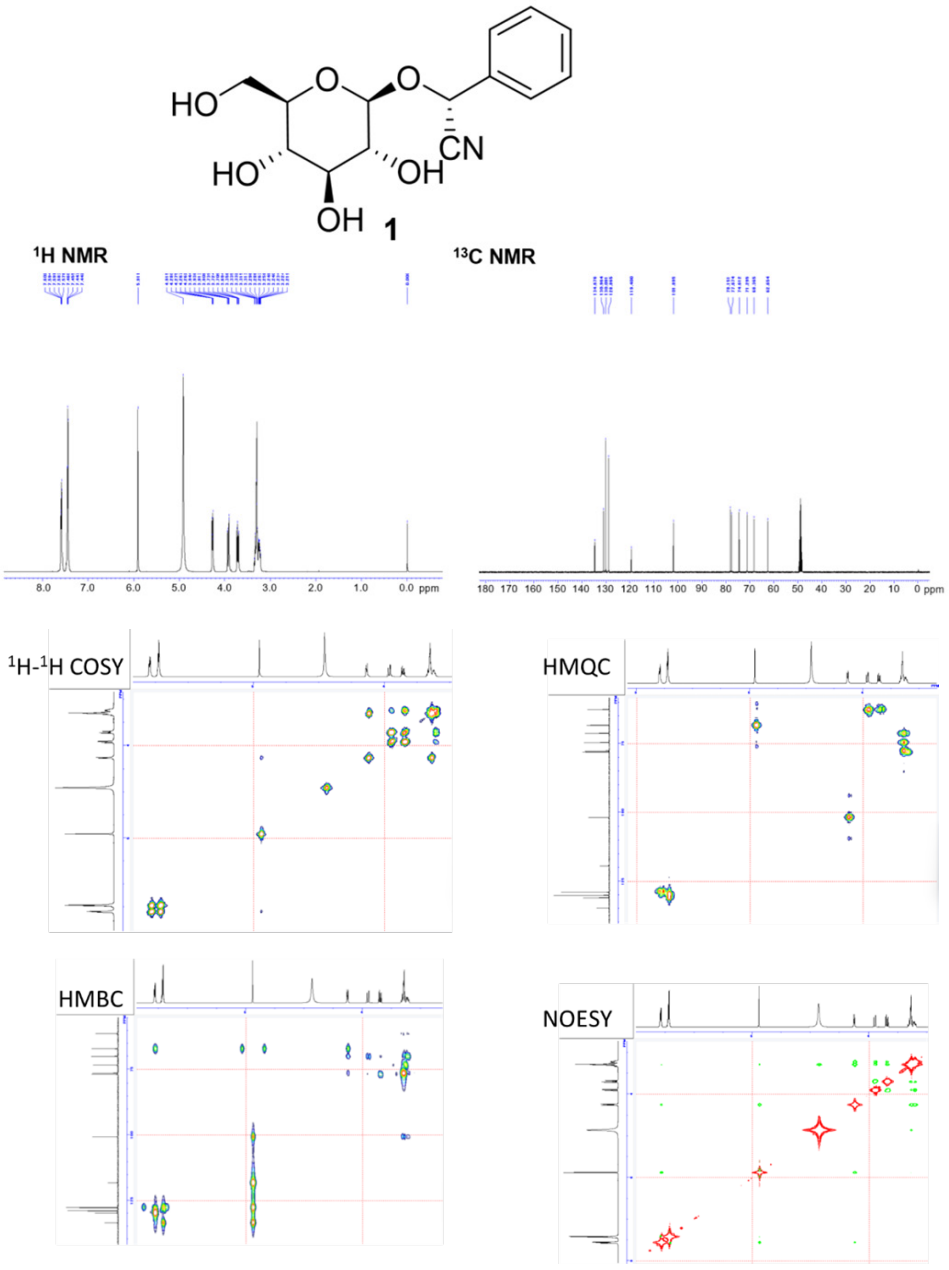

Figure 1. Chemical structure of 1 and its NMR spectra (in methanol-d_4).

Compounds **2**–**5** are typical flavanones (Figure 2). These flavanones were identified as the main constituents of the plant, and **2** and **4** contained a catechol moiety. These compounds were isolated in large quantities in a non-glycosidic form. Given that flavanones have many biological activities in animals, it is of interest to know what effects the compounds contained in this plant, which is ingested as a precious feed in winter, may have.[11]

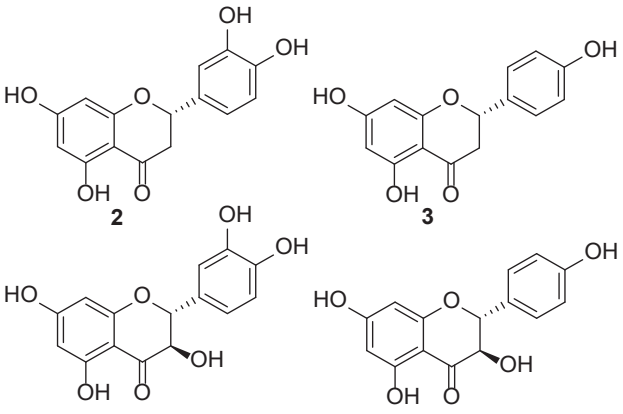

Figure 2. Chemical structures of 2–5.

A. pedunculata is a promising wild almond in terms of its high nutritional value and is expected to be used effectively in the future (Figures 3A and B).[12] Goats and sheep often eat the fruits of this plant (Figure 3C). Its beautiful peach-colored flowers (Figure 3D), which bloom in early spring and adorn mountains and streets, are considered of great value. The flowers of this genus are widely consumed by livestock animals in spring, and are recognized by the local nomads as a good feed to recover the weakened body during the winter season. Furthermore, if the plant is useful as a winter feed for livestock, together with other shrubs and tall grasses (Figure 3E), and its toxicity is not a problem, as pursued in this research, protecting and nurturing the plant in its native habitat will also contribute to environmental conservation in the Mongolian Steppe. Recently, research has been conducted in Arkhust to develop suitable seedlings and procedures for their establishment (Figure 3F).

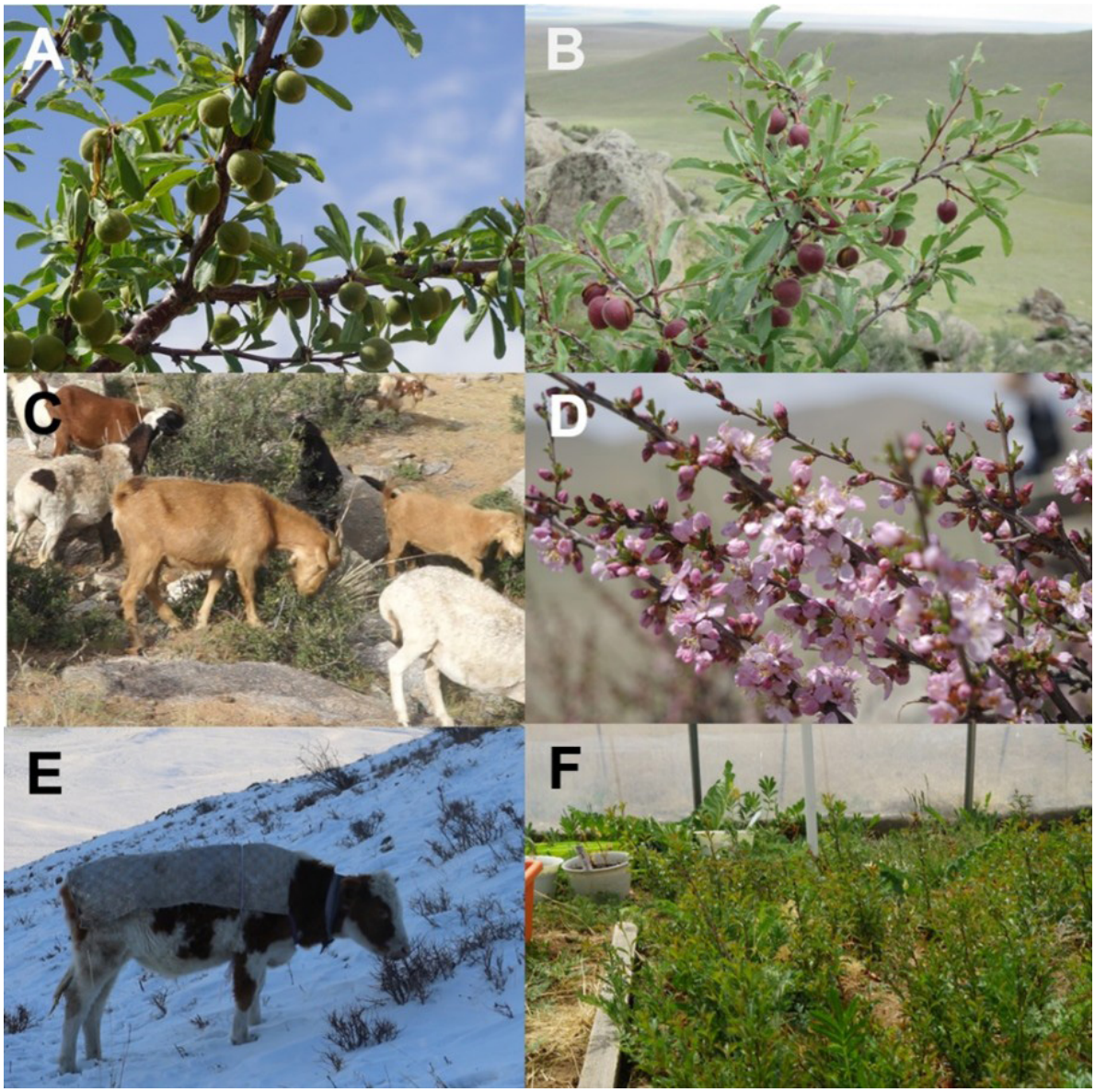

Figure 3. (A) Unripe fruits and shrub of *Amygdalus pedunculata*; (B) Fruits and shrub of *A. pedunculata*; (C) Goats and sheep eating fruits and shrubs of *A. pedunculata*; (D) Flowers of *A. pedunculata*; (E) Cow eating shrubs on the snow; and (F) Attempts to cultivate seedlings of *A. pedunculata*. These photos were taken by B. Batkhuu and B. Batbold.

By conducting fundamental research on the characteristic chemical constituents of plants native to Mongolia and their biological activities, this study aimed to reaffirm their value and contribute to the preservation of both the grassland ecosystem and the livelihoods of local communities.

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