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Chemical Composition and Antioxidant Activity of the Poll Gland Secretions of the Bactrian Camel *(Camelus bactrianus)*

Batjargal Batdorj[1], Munkh-Erdene Purev[2], Tserenjadamba Bayandorj[1], Damdindorj Lkhagvasuren[3], Oyunsuren Tsendsuren[3]

[1]Department of Biology, School of Arts and Sciences, National University of Mongolia, Ulaanbaatar, Mongolia; [2]Department of General Education, National University of Medical Sciences, Ulaanbaatar, Mongolia; [3]Laboratory of Molecular Biology, Institute of General and Experimental Biology, Ulaanbaatar, Mongolia

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Corresponding Author
Batjargal Batdorj, PhD
Department of Biology, School of Arts and Sciences, National University of Mongolia, Ulaanbaatar 14200, Mongolia
Tel: +976-9922-0067
E-mail: batjargal@num.edu.mn

Objectives: To determine chemical composition and antioxidant activity of poll gland secretions. **Methods:** Physical and chemical constituents, such as moisture and ash content, were determined using standard methods, crude fat was determined using the Soxhlet method, and organic composition was analyzed using GC-MS. Macro- and micro- element contents were determined using ICP-OES. Protein concentration was determined using the Bradford assay, and protein profiles were determined using SDS-PAGE. Antioxidant activity was determined using the DPPH free-radical scavenging technique. **Results:** Camel poll gland secretions contained 23 minerals. Potassium (89030 mg/kg) was most predominant, followed by calcium (12955 mg/kg), iron (8546 g/kg), aluminum (6105 mg/kg), magnesium (4563 mg/kg), sodium (2452 mg/kg), and zinc (14198 mg/kg). Total protein content was 11.7%. SDS-PAGE analysis of poll gland secretions revealed the presence of three different protein bands with the molecular weights of 35kDa, 68kDa and 130.5kDa. 12 organic compounds were determined by GC-MS in the poll gland secretions. Antioxidant activity of poll gland secretions was IC 50-176.2 μg/ml. **Conclusion:** Proteins with the molecular weights of 35kDa, 68kDa, and 130.5kDa were determined in poll gland secretions, and they displayed potent antioxidant activity.

Keywords: Camels, SDS-PAGE, Minerals, 2,2-diphenyl-1-picrylhydrazyl

Introduction

The male Bactrian camel *(Camelus bactrianus)* possesses an occipital scent gland which secretes profusely during the winter rutting period. This gland, which is absent in the female, is paired with macroscopically discrete structures overlying the occipital bone of the skull and composed of glandular lobules containing tubuloalveolar cells imbedded in a fibrous aroma [1]. When the testosterone levels are high, there is a large dark brown, acrid smelling secretion from the occipital gland [2].

In Mongolian folk medicine, camel poll gland secretions have been used for the treatment of cancer, particularly

gynecological cancer, however, there is little research on the chemical composition and biological activity of these poll gland secretions. Ayorinde F et al. have used gas chromatography-mass spectrometer to find three methyl butanoic acid methyl ester, hexane, and decane acids from C_{15}-C_{25}, with the exception C_{24} saturated carbon acid, and γ-dodecalactone in the secretion [1]. Khorolmaa et al. have determined some chemical compositions, such as protein, lipid, carbohydrate and testosterone content, in poll gland secretions [3]. Tserendagva et al. have shown that the secretions of poll gland increase thrombocytes [4]. Likewise, Wang noticed that poll gland secretions of the Bactrian camel in rats induced serum levels of immune factors, increased the immunity, and reduced nitric-oxide synthase (NOS) levels of leiomyoma uteri [5]. In Arab countries, there have been studies on the dromedary poll gland secretions, histology, morphology, as well as its protein band [6-8].

Since 1990, cancer has been the second leading cause of population mortality in Mongolia [9]. Thus, there is an increased interest in various forms of cancer treatment, including chemotherapy, hormonal supplements, surgery, radiation therapy, and complementary or alternative medicine [10]. Antioxidants have been shown to prevent the types of free radical damage that have been associated with cancer development. Therefore, researchers have investigated whether taking antioxidant supplements can help lower the risk of developing or dying from cancer in humans [11].

The administration of poll gland secretions to cancer patients without physician guidance is increasing and popularizing in Mongolia. Hence, there is increasing demand for studies on the chemical composition and biological activity of poll gland secretions and their use in cancer treatments. Our aim was to determine the chemical composition and biological activity of poll gland secretions of the male Bactrian camels.

Materials and Methods

The samples were collected from ten different camel breading souls within six aimags during the rutting seasons, which is from December to March, from 2012 to 2014 (Figure 1). Hair samples were clipped from the occipital region from 13 different animals, put into a cellophane bag, and transferred to the laboratory in an ice box. After the samples were cleaned from the wool and mechanical mixture by sorting, settling, and sieving, the sample yield was 36.8±22.08%. The prepared samples of the poll secretions were stored in sterilized tubes at -20^{0}C.

1. Chemical composition and protein profiles

In order to determine the chemical composition of poll gland secretions, the following analyses were undertaken. Physical and chemical constituents, such as moisture and ash content, were determined using standard methods, and crude fat was determined using the Soxhlet method [12, 13]. Protein

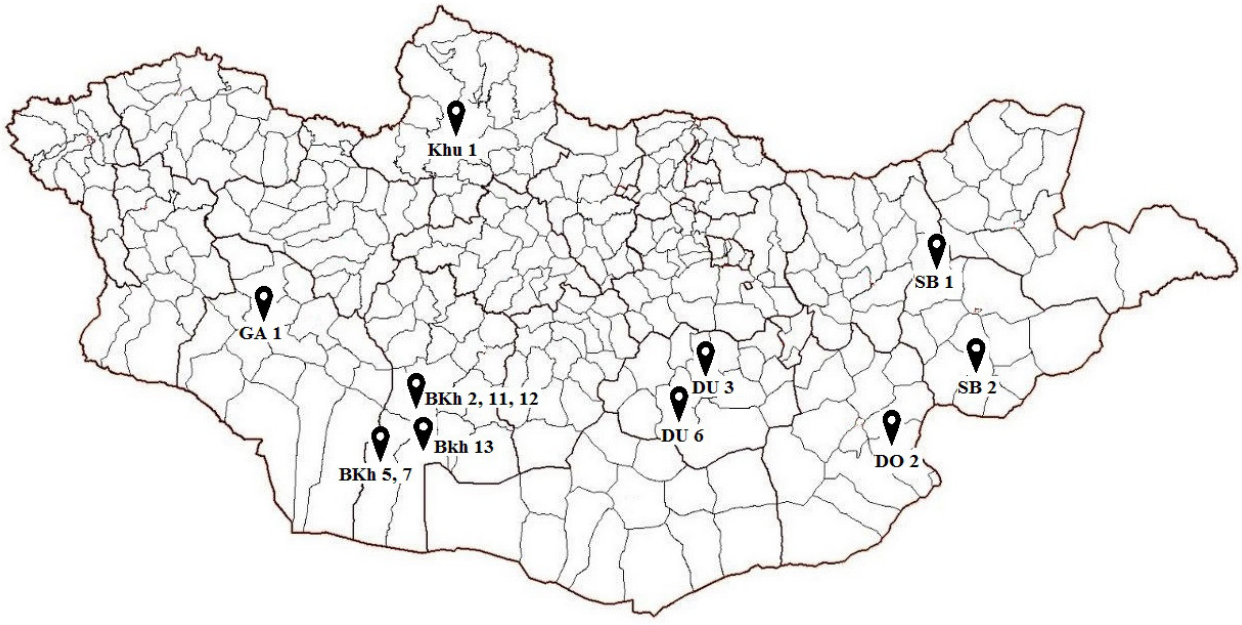

Figure 1. Map of sampling locations

concentration was determined using the Bradford assay, and protein precipitation was conducted using 100% trichloroacetic acid (TCA) in 1/10 volume of sample, as described by Ballog [14]. Concentrated proteins were dissolved in 50mM phosphate buffer and subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel to determine protein profiles [14].

2. GC-MS analysis

In order to determine organic composition, we performed Soxhlet extractions with analytical grade methanol. The extracted solution was analyzed by gas chromatography–mass spectrometry (GC-MS) (Varian CP 3800-1200 Quadrupole, Palo Alto, California) with a column of CP-Sil 8 Low Bleed/MS. For GC-MS detection, helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 µl was employed (split ratio of 20:1). The injector temperature was maintained at 300°C, the ion-source temperature was at 200°C, and the oven temperature was programmed at 70°C (isothermal for 2 min), with an increase of 10°C/min to 230°C, then 8°C/min to 240°C, then ending with a 25 min isothermal at 240°C. Mass spectra were taken at 70 eV. Interpretation on mass-spectrum GC-MS was conducted using the Wiley and Mainlab databases.

3. ICP-OES method

Macro- and micro- element contents were determined using the analytical technique of inductively coupled plasma optical emission spectroscopy (ICP-OES). After acid digestion with aqua regia, an inductively coupled plasma optical emission spectrometer (Varian ES 720) was used to perform a quantitative analysis of 23 types of elements [15].

4. DPPH free-radical scavenging technique

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging technique. First, we prepared precipitated proteins from poll gland secretions with four different concentrations (50mg/ml, 100mg/ml, 200mg/ml, 400mg/ml), then we added a 1 ml solution of 0.004% DPPH (2,2-diphenyl-1-picrylhydrazyl) dissolved in 95% ethanol. The mixture was then shaken and left in a dark space for 30 min at room temperature, and the absorbance of the resulting solution was read at 517 nm. A lower absorbance represented a higher DPPH scavenging activity. The scavenging effect was expressed as shown in the following equation: DPPH scavenging activity (%) = [(control absorbance-sample absorbance)/control absorbance] x 100.

Our control consisted of 1 ml of ethanol and 1ml of 0.004% DPPH [16]. The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity. A lower IC_{50} meant a better radical scavenging activity [17].

5. Statistical analysis

All experiments were repeated three times, and sample means and standard deviations were calculated using MS Excel 2010. Additionally, the correlation coefficient of protein content and antioxidant activity was computed [18].

Results

1. Chemical composition and protein profiles

The poll gland secretion samples used in our study was collected from six different aimags and analyzed to determine its chemical composition. Depending on the condition of the sample, the moisture content ranged from 8.03% to 12.8%, the dry matter content was from 87.2% to 91.97%, the protein content was from 2.8% to 21.1%, and the fat content from 0.75% to 6.8%, ash content ranged from 57.01% to 59.3%. Thus, the average moisture content was calculated to be 9.9%, the dry matter was 90.1%, the protein content was 11.7 %, the fat content was 3.37%, and the ash content was 58.1%, as shown in Table 1. Using SDS-PAGE, we identified the protein bands with the molecular weights of 130.5kDa, 68kDa, and 38kDa in all the samples, thus forming the main protein profile of poll gland secretions, as shown Figure 2.

2. Result of GC-MS analysis

We identified 12 substances using GC-MS analysis, including saturated and aromatic hydrocarbons, ketones, fatty acid esters, amines, amino acids, carbohydrates and alkaloids, as listed in Table 2. The poll gland secretion GC-MS chromatogram is shown in Figure 3. Among the 12 substances found, 1-acetyl-16-methoxy-aspidospermidin-17-ol, an alkaloid with the molecular weigh of 370, was determined to hold much interest.

3. Result of macro, micro elements content analysis

Table 1. Moisture, dry matter, protein, fat, and ash content of poll gland secretions

Constituents	Content (mean±SD)
Moisture	9.9±1.72
Dry matter	90.1±1.72
Protein	11.7±5.23
Fat	3.37±2.15
Ash	58.1±2.21

A total of 23 element concentrations were measured using ICP-OES and have been illustrated in the Table 3. We found high levels of calcium (12955.7 mg/kg), iron (8546.9mg/kg), magnesium (4563.8 mg/kg), and sodium (2452.5 mg/kg). Heavy metals, such as cadmium (1.1 mg/kg), lead (2.5 mg/kg), and beryllium (0.38 mg/kg), were observed in low levels.

4. Antioxidant activity of poll gland secretion

The poll gland secretions displayed a potent antioxidant activity with an IC_{50} value of 176.2 µg/ml. Among all samples, sample GA 1 showed highest antioxidant value of IC_{50}-60.7 µg/ ml, and sample Khu 1 showed the lowest activity (IC_{50}-339 µg/ml), as shown in Table 4. The antioxidant activity increased depending on protein concentration. The correlation coefficient of protein content and antioxidant activity IC_{50} value was -0.75, and thus, we can conclude that the protein concentration and antioxidant activity have a negative linear relationship.

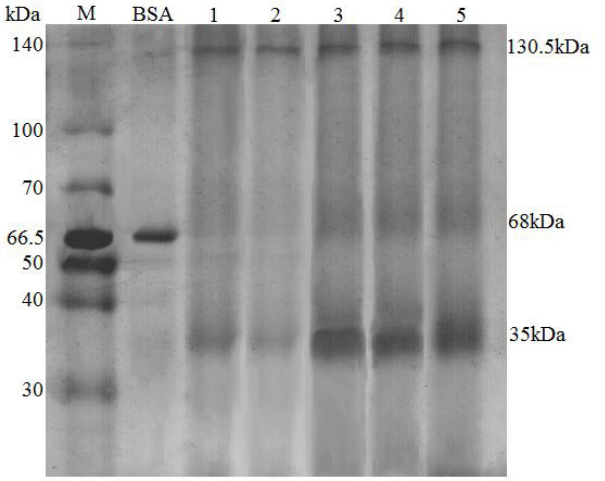

Figure 2. SDS-PAGE (12.5%, Comassie blue staining) of poll gland secretions

M-Mid-Range pre-stainedrecombinant protein marker (LandMark™); BSA-Bovine serum albumin (Carl Roth); 1-Sample from Bayarntsagaan sum, Bayankhongoraimag (Bkh 2); **2**- Sample from Dornogobiaimag (Do2); **3**-Sample from Tumentsogt sum, Sukhbaataraimak (SB1); **4**- Sample from Bayarntsagaan sum, Bayankhongoraimag (Bkh11); **5**- Sample from Ongon sum, Sukhbaataraimag (SB2)

Discussion

The medicinal use of natural products—compounds that are derived from natural sources such as plants, animals, or microorganisms—precedes recorded human history by thousands of years [19]. In the past few years, we have seen a renewed interest in the use of camel products for its therapeutic benefits. The long-standing practice of using camel milk and

Table 2. Components of poll gland secretion found using GS-MS

Substances	Formula
Toluene	C_7H_8
3-penten-2-one, 4-methyl	$C_6H_{10}O$
Benzene, ethyl	C_8H_{10}
Decane	$C_{10}H_{22}$
Decane, 2-methyl	$C_{11}H_{24}$
Alanine, 3-(benzyloxy)-,L-	$C_{10}H_{13}NO_3$
Tetradecane, 2,6,10-trimethyl	$C_{17}H_{36}$
3-amino-4-[(1-benzyl-2-methoxy-2-oxoethyl)amino]-4-oxobutanoic acid	$C_{14}H_{18}N_2O_5$
Alpha-D-mannofuranosid, 1-o-decyl-	$C_{16}H_{32}O_6$
Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$
Neronine, 4. beta., 5-dihydro	$C_{18}H_{21}NO_6$
Aspidospermidin-17-ol,1-acetyl-16- Methoxy	$C_{22}H_{30}N_2O_3$

Table 3. Mineral composition of poll gland secretions

Elements	Symbol	Content (mg/kg)
Silver	Ag	10.7
Aluminium	Al	6105
Arsenic	As	15.7
Boron	B	7.2
Barium	Ba	81.3
Berillium	Be	0.38
Calcium	Ca	12955.7
Cadmium	Cd	1.1
Cobalt	Co	6.9
Chromium	Cr	10.7
Copper	Cu	18.8
Iron	Fe	8546.9
Potassium	K	89030.5
Magnesium	Mg	4563.8
Manganese	Mn	215.9
Molybdenum	Mo	5.8
Sodium	Na	2452.5
Nickel	Ni	6.6
Lead	Pb	2.5
Antimony	Sb	97.7
Selenium	Se	128.7
Zirconium	Zr	54.5
Zinc	Zn	1198.2

Table 4. Protein content and DPPH radical scavenging activity of the poll gland secretions

Sample number	Protein content %	IC_{50} (μg/ml)
BKh 2	12.7±0.68	158.4±8,9
BKh 5	8.5±2.57	241.5±3.5
BKh 7	2.8±0.58	320±5.08
BKh 11	21.1±0.70	106.9±4.8
DO 2	17.1±2	140±7.6
Khu 1	4.1±1.2	339±15.0
GA 1	12±1.22	60.7±10.4
SB 1	11.1±1.02	131.9±6.9
BKh 12	15.8±0.75	182.2 ±6.3
BKh 13	9±0.43	113.9±8.4
DU3	11.6±0.15	183.4±13.5
DU 6	9.25±0.92	215±27.1
SB 2	17.3±0.88	98.7±4.3
Correlation coefficient, r	-0.75	

A lower IC_{50} means better radical scavenging activity. The correlation coefficient of protein content and antioxidant activity (IC_{50} value) was -0.75.

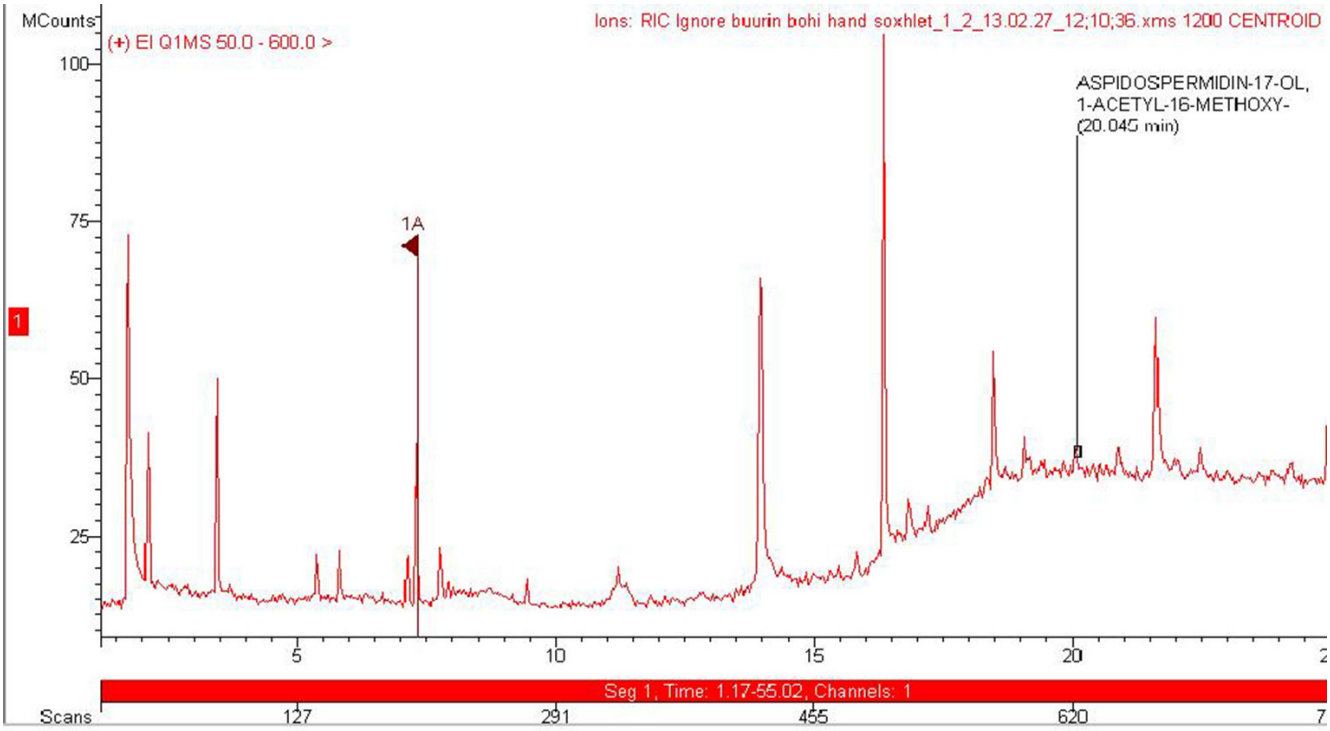

Figure 3. GC-MS chromatogram of poll gland secretions

urine for medicinal purposes in the Middle East, parts of Africa and Asia, and the former Soviet Union was without scientific rationale for centuries [20]. In Mongolian folk medicine, camel poll gland secretions have been used for the treatment of cancer, particularly gynecological cancer, however there is little research on the chemical composition and biological activity of poll gland secretions.

Previously, F.Ayorindeet et al. determined three methyl butanoic acid methyl ester, hexane, and decane acids from C_{15}-C_{25}, with the exception of C_{24} saturated carbon acid, and γ-dodecalactone using GC-MS [1]. In our study, we found 12 substances in our poll gland secretions using GC-MS analysis. Among the 12 substances, 1-acetyl-16-methoxy-aspidospermidin-17-ol was noteworthy because aspidospermidine related alkaloids have been used in breast, cervical, and lung cancer chemotherapy [21]. For example, one aspidospermidine related alkaloid is vinorebline, and vinblastine derivative semi-synthetic compounds have been used for breast and lung cancer chemotherapy [22].

In our study, we determined a general chemical composition of poll gland secretions. Results showed that average moisture, dry matter, protein, fat, and ash content in poll gland secretions were 9.9 %, 90.1%, 11.7%, 3.37%, and 58.1%, respectively. Our protein content of 11.7% was similar to levels previously determined by Khorolmaa et al. [3]. Using SDS-PAGE methods, we identified protein bands with the molecular weight of of 130.5 kDa, 68kDa, and 38 kDa in all our samples, thus forming the main protein profile in poll gland secretions. Ebada et al. analyzed poll gland secretion proteins for one-humped camel and found actin (42kDa), cytokeratin (45kDa), and S100 (14kDa) [8]. Our results differed from protein bands found in one-humped camels.

Recently, bioactive peptides and proteins have received close scientific attention for their broad scope of bioactivities, mainly including anti-oxidation, antihypertensive, anticancer, and antimicrobial properties [23, 24]. It has been reported that bioactive peptides may originate from various natural proteins, such as cereals, legumes, milk, meat, egg, fish, and various marine organisms [25, 26]. In fact, the search for novel bioactive peptides has increased considerably in the last few decades. In our study, we determined that partially purified protein extraction of poll gland secretions displayed potent antioxidant activity.

Antioxidant peptides have been widely studied for their significant prevention and improvement of conditions arising from non-communicable chronic degenerative diseases [24]. During an organism's metabolism, reactive oxygen species (ROS) or free radicals are naturally produced through oxidation reactions during breathing. Living organisms have developed their own antioxidant defenses against excessive amounts of ROS. However, when their limited efficiencies cannot prevent all the oxidative damages related to environmental conditions (i.e., UV light, non-equilibrated food, and pollution), the excessive amounts of ROS cause oxidative stress, which results in many non-communicable chronic diseases, such as diabetes, atherosclerosis, arthritis, and cancer [27-29]. Natural antioxidants have been found to possess the ability to effectively prevent the damage caused by ROS [30]. Therefore, there is a growing interest in exploring various antioxidant natural compounds, such as peptides derived from natural proteins.

To the best of our knowledge, this is the first report on the protein profile and antioxidant acitivity of poll gland secretions. A limitation of this study was the small amount of the samples due to difficulty in obtaining samples and the cost of the field work. Nomadic families typically only have one male camel for breeding, and during rutting season, male camels are very aggressive, making it difficult to catch them and clip hair from occipital region.

The antioxidant properties of proteins of the poll gland secretion may be prominent to human health, and thus, future studies should focus on purifying proteins from poll gland secretions to test its anticancer and apoptotic effects on breast (HCT1937) and liver cancer (HepG2) cells and breast immortal (MCF10A) cell lines.

Conflict of Interest

The authors state no conflict of interest.

Acknowledgements

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