

## Deer Antler Extracts: Extraction Methods and Functional Properties

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### Abstract

For centuries humans have been interested in using biologically active compounds of animal origin to supplement their diets and to prevent and treat diseases. Nowadays, many extracts are produced to ensure the availability of dietary supplements to consumers worldwide. However, with the population constantly growing, there is a need to develop more effective extraction methods that can enable producers to supply the global market with high-quality products. In this paper, we study the structures and the chemical compositions of antler extracts prepared with antlers of the Altai wapiti and the Tundra Reindeer and compare conventional percolation with a circular (recurring) percolation method that involves multiple, repeated usage of the solvent. We also examine the concentration of the solvent. We argue that circular percolation provides dietary supplement producers with more opportunities, as with this method, the obtained extract is richer in biologically active compounds. The findings of the study can be of interest to dietary supplement producers.

**Keywords:** Antler extract, Extraction method, Percolation, Pantohepatogen

### Introduction

In our contemporary, fast-paced environment dietary supplements are gaining popularity. Extensive studies have demonstrated that dietary supplements offer a gentle, but effective and long-lasting influence on metabolic processes; therefore, they are increasingly

used to maintain good nutrition and health (Pokrovskij *et al.*, 2002; Provalova *et al.*, 2002; Zhdanov *et al.*, 2005; Guryanov, 2010; Dorn *et al.*, 2013; Dygai *et al.*, 2013; Guryanov, 2013; Dorn *et al.*, 2014; Mirmiran *et al.*, 2014; Flodin *et al.*, 2015; Poznyakovsky *et al.*, 2017; Vekovtsev *et al.*, 2017; Buscemi *et al.*, 2018; Zhang *et al.*, 2018; Eksi *et al.*, 2019; Tokhiriyon & Poznyakovsky, 2019; Tokhiriyon *et al.*, 2019; 2020; Derbyshire *et al.*, 2021; Sergun *et al.*, 2021). Discussing dietary supplements produced with ingredients of animal origin, we would like to focus on antler supplements, in particular, pantohepatogen. Pantohepatogen is produced using antlers of the Altai wapiti (*Cervus elaphus sibiricus*); the Manchurian wapiti (*Cervus elaphus xanthophigus*); the Sika Deer (*Nippon hortulorum Pseudaxis hortulorum*); and the Tundra Reindeer (*Tarandus rangiferi*). Antler products have been used in traditional medicine for centuries, and recent studies have identified how dietary supplements influence and modulate body processes (Goldberg *et al.*, 2000; Duchateau & Klaffke, 2009; Guryanov, 2010, 2013; Ferguson *et al.*, 2013).

Velvet antlers are young, tender horns that consist of cartilage and bone and are filled with blood. Velvet antlers are of great value as no other animal can demonstrate bone growth as fast as that of deer species. This high growth rate requires significant effort and, thus, antler regeneration involves all functional systems.

Depending on the type of deer, there are certain differences in the composition of antler products. The most valuable antlers belong to the Altai wapiti. In the Russian Federation, the Altai wapiti is found in high mountain areas with favorable, clean environments. The Altai wapiti's eating patterns are selective; animals mainly consume golden root, maral root, and other endemic plants with known medicinal properties. Biologically active substances obtained with food and mixed with blood are accumulated in antlers. Antlers are harvested annually, in autumn, during the rutting period.

### Materials and Methods

We studied and compared the structures and the chemical compositions of the Altai wapiti antlers, the Manchurian wapiti antlers, the Sika Deer antlers, and the Tundra Reindeer antlers, and also analyzed the dietary supplements that contain antlers as active ingredients. During the chemical analysis, we determined five main groups of substances: mineral substances, amino acids, peptides, lipids, and nucleic acids.

We also ascertained that the functional properties of antler products hugely depend on the extraction method. This led us to

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question the traditional extraction method which is percolation. This method involves filtering a solvent (an extractant) through raw material in order to pull out desirable substances soluble in the solvent. Traditionally, the saturated solvent is replaced by the fresh solvent. We argue for circular (recurring) percolation which involves multiple, repeated usage of the solvent.

## Results and Discussion

**Table 1.** Comparison of the extraction methods and the extracted ingredients

Indicators	Extraction method	
	Percolation 40% ethanol	Circular (recurring) percolation 70% ethanol
Extracted ingredients	3.41	11.68
	<b>Including:</b>	
Proteins	1.52	4.15
Biologically active mass	1.89	7.53
Lipids	0.053	0.40

Proteins extracted with a 40% ethanol solvent firm up when being cooled and turn into a jelly-like texture, which makes it more difficult to filtrate the extract. If a 70% ethanol solvent is used, then partial denaturation takes place and, during further processing, protein precipitation occurs.

As can be seen from the table, when the traditional percolation method with a 40% ethanol solvent is used, only 11% of lipids are extracted. While the application of the circular (recurring) percolation method with an acidified 70% ethanol solvent results in up to 84% of lipids being extracted. Therefore, we can conclude that circular (recurring) percolation with a 70% ethanol solvent is more beneficial for obtaining larger quantities of desirable ingredients.

During our study, we applied both traditional percolation and circular (recurring) percolation to extract the active ingredients from antlers. When compared, the findings indicated a 3.5 times difference in the quantity of the extracted ingredients (**Table 1**). Additionally, the higher concentration of the solvent results in a larger quantity of the extracted ingredients, while lower concentrations mainly extract protein. In particular, when 40% ethanol solvent is filtered through antlers, protein amounts to 44.7 % of the extract.

The comparison of active ingredients in antler products made from antlers of the Altai wapiti, the Manchurian wapiti, the Sika Deer, and the Tundra Reindeer did not demonstrate any significant differences in the quantity of active ingredients. The chemical compositions of two antler extracts prepared with antlers of the Altai wapiti and the Tundra Reindeer are presented in **Table 2**. However, the concentration of active ingredients depends on the quality of the raw material, and the time and location of harvesting.

The information about the hormones found in the Tundra Reindeer extract is presented in **Table 3**.

**Table 2.** The chemical compositions of two antler extracts

Indicators	'Velkornin' the Tundra Reindeer extract	'Pantokrin' the Altai wapiti extract
Density	0.90 – 1.00	0.90 - 1.00
Dry matter content, %	0.80 – 0.90	0.65 – 0.80
Alcohol content, %	65 – 70	65 – 70
Organic matter, % including:		
Protein, %	0.0005 – 0.0001	0.001 – 0.0005
Glycogen, mg / 100 g	6.5 – 7.0	3.5 – 4.5
Total sugar, mg / 100 g	4.0 – 5.0	3.0 – 4.0
Choline esters, mg / 100 g	1.10 - 1.20	1.5 – 1.7
Amino acids, mg / 100 g	125 - 150	150 - 180
Lipids, mg / 100 g	0.12 – 0.15	0.15 – 0.20
Ash, mg / 100 g	0.05	0.05

**Table 3.** The hormones found in the Tundra Reindeer extract

Triiodothyronine (T <sub>3</sub> ), nmol/L	Thyroxine (T <sub>4</sub> ), nmol/L	Cortisol, nmol/L	Estradiol, nmol/L	Progesterone, nmol/L	Testosterone, nmol/mL
5.84	67.00	74.42	0.76	0.48	61.35

The analyses of the mineral content revealed significant amounts of sodium, calcium, and magnesium as well as small traces of aluminum, silicon, copper, iron, and manganese. The sensitivity of the method used did not allow for the detection of other metals. The data on toxicological and microbiological safety are presented

in **Tables 4-6**. The findings of the analyses prove that the content of the active ingredients (with measurement errors taken into account) corresponds to the data provided by the producers of antler products.

**Table 4.** Biologically active components

Components	Content
Iron, mg/kg	252.0
Ascorbic acid, mg / caps	4.6

**Table 5.** The data on the toxicological safety

Indicators	Content, mg/kg
Toxic elements:	
Lead	<0.03
Cadmium	<0.004
Arsenic	<0.001
Mercury	<0.005
Harmful substances:	
HCH isomers	not found
DDT metabolites	not found
Aldrin	not found
Heptachlor	not found
Chloramphenicol	not found

**Table 6.** The data on the microbiological safety

Indicators	Content
Mesophilic aerobic and facultative-anaerobic microorganisms, CFU in 1 g of the product	40
BCGP (coliforms), the tested sample weight - 0.1	not found
Pathogenic, salmonellae included, the tested sample weight – 10 g	not found
Yeast, CFU / g	< 5
Mesophilic sulfite-reducing clostridia, the tested sample weight - 0.1	not allowed
E.coli, the tested sample weight – 1 g	not allowed
S.aureus, the tested sample weight – 1 g	not allowed
Moulds, CFU / g	< 10

## Conclusion

The findings of our study indicate that antler extracts are rich in biologically active compounds, including proteins, amino acids, lipids, carbohydrates, minerals, and hormones, which can influence body functions as well as provide nutritional value. And with the help of circular (recurring) percolation, it is possible to obtain a more saturated extract. The findings of the present study are valuable for producers of dietary supplements, including those developing new dietary supplements and confectionary products with pantothenic acid. However, further research is necessary to

examine the influence of antler supplements and to evaluate their efficacy, functional properties, and process technologies.

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**Ethics statement:** The study was conducted according to the guidelines of the Declaration of Helsinki.

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