



DETERMINATION OF THE CHEMICAL COMPOSITION OF THE PLANT *ARNICA MONTANA*

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ABSTRACT

Arnica montana (also called wolf's bane) is a tall perineal plant with large yellow flowers that belongs to the daisy family Asteraceae and is widespread across Europe. *Arnica montana* is used in small oral doses in homeopathic medicine and in low doses as treatment for pain, inflammation and fever, most often post-surgery or trauma. It is also used more conventionally as a topical medication for wound healing, swelling, inflammation, bruising and pain. However, there is little evidence for its efficacy either as an oral homeopathic or a topical herbal therapy. The FDA classifies *Arnica montana* as an unsafe herb and cautions against using it orally or applied to broken skin where absorption can occur. *Arnica montana* extracts have antiinflammatory effects *in vitro* and have been purported to be beneficial for pain and swelling when applied topically. The clinical studies have not confirmed its effects on inflammation or pain. *Arnica montana* is available in multiple over-the-counter topical forms which are claimed to be useful in treating painful skin conditions. *Arnica montana* extracts applied topically have mild-to-moderate adverse events, which usually overwhelm any beneficial effects they may have on skin conditions. Oral administration of *Arnica montana* in moderate amounts can cause gastrointestinal upset, nausea, vomiting and abdominal pain. *Arnica montana* extracts can cause skin rash and urticaria and should not be taken by mouth.

Introduction

Arnica montana (*A. montana*) has been used for centuries in the homeopathic system of medicine for the treatment of 66 pathological conditions, frequently contusions, wounds, rheumatism, and inflammation. According to European *Pharmacopeia* (1809), *A. montana* tincture is produced from *A. montana* flowers (Fig. 11.2) with 0.04% sesquiterpene



lactones expressed as dihydrohelenalin tiglate. The tincture contains one part of the drug in 10 parts of ethanol [60% (V/V) to 70% (V/V)]. According to the European Union, herbal preparations containing *A. montana* are tincture (1:10) extracted with ethanol 70% (V/V), tincture (1:5) extracted with ethanol 60% (V/V) and liquid extract (1:20) extracted with ethanol 50% m/m, mainly of flowers. Tincture is dried by evaporation, and the extract is incorporated in numerous herbal drug products. destroyed by an evil sorceress, whose nail sprouted a beautiful golden flower, which continues to this day to heal people from various diseases [4] Arnica was used primarily for bruises (e.g., 1 oz arnica tincture combined with 2 oz water; soak a cloth and apply topically) (McClure, 1917). Another formula recommended by Dadd (1854) consisted of 4 ounces arnica flowers in 1 pint new rum. This was macerated for 14 days; then, 1 ounce in a pint of water was used topically for all wounds, bruises, and saddle galls. A sedative drench was also made by Dadd for internal use, to decrease arterial "actation" (i.e., increased pulse rate): 4 drachms (14.8 mL) arnica mixed in 1 pint water; this was repeated as needed, but gradually, the dose was lessened.

Modern use

Arnica is used commonly in topical liniments or ointments for bruises, sprains, and contusions. Even more common than its topical use is its homeopathic use, in which the diluted preparation is taken internally to treat bruises. Internally, it is toxic in the herbal form (Brinker, 2001) but safe in the homeopathic preparation. In Europe, the internal form of arnica is banned (Brinker, 2000). The following is the topical formula for bruises: brew handful of flowers or whole plant as a tea in 2 cups water (not to be strained); then, massage onto injured parts (deBairacli-Levy, 1976).

Materials and methods: Determination of the amount of total protein

The method consists of determining nitrogen using the Kjeldahl method, followed by conversion to protein. The essence of the method is the decomposition of the organic matter of the sample with boiling concentrated sulfuric acid with the formation of ammonium salts, the conversion of ammonium into ammonia, its distillation into an acid solution, the quantitative accounting of ammonia by the titrimetric method and the calculation of the nitrogen content in the material under study.

From an average crushed homogeneous sample of the studied low-fat cotton seed meal, an accurate sample was weighed in a test tube for analysis, with an error of no more than 0.1%. The sample was quantitatively transferred to a Kjeldahl flask. Further experiments were carried out according to the methodological instructions [10].

Instruments and utensils used: ISPMSNEXION-2000 or similar mass spectrometer,

microwave digestion device (Germany) or similar Teflon autoclaves volumetric flasks

Reagents used: multi-element standard No. 3 (29 elements for MS)

standard for -Hg (mercury) nitric acid (chemical/h) hydrogen peroxide (chemical/h)

double-distilled water argon (gas purity 99.995%)

Results: Processing of results: The mass fraction of nitrogen (X) in the test sample as a percentage of its mass during the distillation of ammonia into sulfuric acid was calculated using the formula



$$X = \frac{(V_1 - V_0) \times K \times 0,0014 \times 100}{M}$$

V_0 – volume of 0.1 mol/l sodium hydroxide solution consumed for titration of 0.05 mol/l sulfuric acid in the control experiment, ml. V_1 – volume of 0.1 mol/l sodium hydroxide solution consumed for titration of sulfuric acid in the test solution, ml; K – correction to the titer of 0.1 mol/l sodium hydroxide solution;

0.0014 – amount of nitrogen equivalent to 1 ml of 0.05 mol/l sulfuric acid solution;

M is the mass of the sample, g. The arithmetic mean of the results of five parallel tests was taken as the final test result. Results were calculated to the third decimal place and rounded to the second decimal place.

The mass fraction of nitrogen in terms of dry matter of the product (X_3), in per cent, was calculated using the formula:

$$X_3 = \frac{X_1 \times 100}{100 - W}$$

X_1 – mass fraction of nitrogen in the test sample, %; W – humidity of the test sample, %.

The mass fraction of protein (Y) as a percentage was calculated using the formula: $Y = K \times X$, where K is the conversion factor of nitrogen to protein: with moderate lipid content - 6.38;

Table 1

Protein content of *Calendula officinalis*

Nº	Sample	Nitrogen(%)	Protein(%)
1.	<i>Arnica montana</i>	1,238	14,328

Method for quantitative determination of micro and macroelements using inductively coupled plasma mass spectrometry (ICP-MS)

An accurate sample of 0.0500-0.5000 g of the test substance is weighed on an analytical balance and transferred to Teflon autoclaves. Then the autoclaves are filled with the appropriate amount of purified concentrated mineral acids (nitric acid (h/h) and hydrogen peroxide (h/h)). The autoclaves are closed and placed on a Berghofc microwave digestion device using the MWS-3+ software or a similar type of microwave digestion device. Determine the decomposition program based on the type of substance under study, indicate the degree of decomposition and the number of autoclaves (up to 12 pcs). After decomposition, the contents in autoclaves are quantitatively transferred into 50 or 100 ml volumetric flasks and the volume is adjusted to the mark with 0.5% nitric acid. The determination of the substance under study is carried out using an ISPMS device or a similar optical emission spectrometer device with inductively coupled argon plasma. In the determination method, the optimal wavelength of the micro or macroelements being determined is indicated, at which they have maximum emission. When constructing a sequence of tests, indicate the amount in mg and the degree of its dilution in ml. After receiving the data, the true quantitative content of the substance in the test sample is automatically calculated by the device and entered in the form of mg/kg or $\mu\text{g/g}$ with error limits - RSD in%.

table 2

Quantitative determination of micro content



and macroelements using the ISPMS method

No	Element	Quantitative content mg/kg	No	Element	Quantitative content mg/kg
1	Silver, Ag	0,009	17	Magnesium, Mg	5463,63
2	Aluminium, Al	0,436	18	Sodium, Na	8347,03
3	Arsenic, As	0,023	19	Manganese, Mn	63,12
4	Barium, Ba	56,17	20	Nickel, Ni	0,002
5	Beryllium, Be	0,008	21	Rubidium, Rb	3,35
6	Bismuth, Bi	0,003	22	Selenium, Se	0,324
7	Calcium, Ca	3068,9	23	Strontium, Sr	45,85
8	Cadmium, Cd	0,019	24	Thallium, Tl	0,001
9	Cobalt, Co	0,067	25	Uranium, U	0,001
10	Chromium, Cr	3,25	26	Vanadium, V	0,08
11	Copper, Cu	7,89	27	Zinc, Zn	8,12
12	Iron, Fe	159,28	28	Lead, Pb	0,001
13	Gallium, Ga	0,75	29	Cesium, Cs	0,002
14	Indium, In	0.001	30	Mercury, Hg	7,113
15	Potassium, K	6006,08	31	Phosphorus, P	6977,3
16	Lithium, Li	0,812	32	Bor V	7,65

Discussion: Based on the obtained results the plant *Arnica montana* has right amount of proteins as well as some micro and macro elements such as Calcium, Potassium and magnesium which help for different part of body to digest and work properly. And this material can be used for future medicinal usage because of its chemical contents.

References:

1. Zimmerman HJ. Unconventional drugs. Miscellaneous drugs and diagnostic chemicals. In, Zimmerman, HJ. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. 2nd ed. Philadelphia: Lippincott, 1999: pp. 731-4..
2. Liu LU, Schiano TD. Hepatotoxicity of herbal medicines, vitamins and natural hepatotoxins. In, Kaplowitz N, DeLeve LD, eds. Drug-induced liver disease. 2nd ed. New York: Informa Healthcare USA, 2007, pp. 733-54.
3. Tveiten D, Brusset S. Effect of Arnica D30 in marathon runners. Pooled results from two double-blind placebo controlled studies. Homeopathy. 2003;92:187-9.
4. Gazim ZC, Rezende CM, Fraga SR, Dias Filho BP, Nakamura CV, Cortez DAG. Analysis of the essential oils from *Calendula officinalis* growing in Brazil using three different extraction procedures. Revista Brasileira de Ciências Farmacêuticas. 2008; 44(3): 391-395.
5. Shahane, K., Kshirsagar, M., Tambe, S., Jain, D., Rout, S., Martins Ferreira, M. K., Mali, S., Amin, P., Srivastav, P. P., Cruz, J., & Lima, R. R. (2023). An Updated Review on the Multifaceted Therapeutic Potential of *Calendula officinalis* L. Pharmaceuticals, 16(4). <https://doi.org/10.3390/ph16040611>



6. Butnariu M, Coradini CZ. Evaluation of Biologically Active Compounds from *Calendula officinalis* Flowers using Spectrophotometry. *Chem Cent J*. 2012 Apr 27;6:35. doi: 10.1186/1752-153X-6-35. PMID: 22540963; PMCID: PMC3379952.
7. Ak, G., Zengin, G., Ceylan, R., Mahomoodally, M. F., Jugreet, S., Mollica, A., & Stefanucci, A. (2021). Chemical composition and biological activities of essential oils from *Calendula officinalis* L. Flowers and leaves. *Flavour and Fragrance Journal*, 36(5), 554-563. <https://doi.org/10.1002/ffj.3661>
8. Arora D, Rani A, Sharma A. A review on phytochemistry and ethnopharmacological aspects of genus *Calendula*. *Pharmacogn Rev*. 2013; 7(14): 179.
9. Safdar W, Majeed H, Naveed I, et al. Pharmacognostical study of the medicinal plant *Calendula officinalis* L. (family Compositae). *Int. J Cell Mol Biol*. 2010; 1: 108-116.
10. Control methods. Chemical factors. Guide to methods of quality control and safety of biologically active food additives. Manual R 4.1.1672-03. M.: Federal Center for State Sanitary and Epidemiological Supervision of the Ministry of Health of Russia, 2004.