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Anatomy and phytochemistry of the seeds of the medicinal and ornamental plant, *Vaccaria hispanica* (Mill.) Rauschert

G'ulom Sh. Jumaboev*, Trobjon Kh. Makhkamov*,,***,◆ and Dilfuza B. Berdibaeva******

**Department of Forestry, Tashkent State Agrarian University, 2 A., Universitet Str., Kibray-District-100700, Tashkent Region, Uzbekistan*

*** Department of Botany and Plant Physiology, National University of Uzbekistan named after Mirzo Ulugbek, 4, Universitet Str.-100174, Tashkent, Uzbekistan*

**** Department of Medicinal Plants and Botany, Gulistan State University, 4, Micro-District, Gulistan-120100, Sir-Darya Region, Uzbekistan*

***** Tashkent Botanical Garden at the Institute of Botany Academy of Sciences of the Republic of Uzbekistan, Uzbekistan*

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Abstract

This article examines the anatomy and phytochemistry of seeds collected from the plantation of *Vaccaria hispanica* (Mill.) Rauschert, which is cultivated as a medicinal crop in Uzbekistan. *V. hispanica* is a weed that is found in the plains, hills, low and middle mountainous areas of Uzbekistan, in fields, neglected and abandoned areas, on the banks of canals, pastures, and is not used for a specific purpose. The seed coat is composed of rectangular and oval-shaped cells and is densely packed. The thickness of the seed coat is 7-10% of the total. Cow cockle germs consist of an embryo (35-40% of seed weight) which nearly encircles the starchy endosperm (*e.g.*, 55-60% of seed weight). Seeds of *V. hispanica* which cultivated in Uzbekistan contain 4.6% fat. *V. hispanica* seeds were determined to contain 13.6% protein, 11.18 mg/g flavonoids and 18.7185 mg/g free amino acids.

1. Introduction

Determining the useful products of a weed species, followed by targeted planting and harvesting, facilitates the conversion of these weeds into crops (Sauer, 1950; Anderson, 1952). According to Vavilov (Vavilov, 1951), before domestication, both rye (*Secale croale*) and oats (*Avena sativa*) were weeds and eventually entered the process of domestication as useful plants.

Vaccaria hispanica (Mill.) Rauschert (synonyms: *Saponaria vaccaria* L.; *Vaccaria pyramidata* Medik.;*Vaccaria segetalis* (Neck.) Garcke ex Asch.; *Vaccaria vulgaris* Host; *Gypsophila vaccaria* Clarke ex Towns) is a member of the Caryophyllaceae family. The family of Caryophyllaceae contains 104 genera with more than 2000 species. Unusual characteristic of this family is the stable and endurable foam that appears, when parts of plants are put into water and shaken (Bottger and Melzig, 2011). *Vaccaria hispanica*–"Ispanmingboshi", "Qoramug" in Uzbek, is an annual herbaceous plant (Vvedenskiy, 1953), an invasive plant that entered the flora of Uzbekistan from Europe. *V. hispanica* is a transformer plant that has overcome all barriers of invasion, expanding its range from day-to-day.

V. hispanica is a weed that is found in the plains, hills, low and middle mountainous areas of Uzbekistan, in fields, neglected and abandoned areas, on the banks of canals, pastures, and is not used for a specific purpose. The natural range of the plant is North Africa, temperate Asia and Europe. In addition, this species has been recorded as an invasive plant in many parts of South Africa, temperate and tropical Asia, Australia, North and South America (Elias, 2006).

Corresponding author: Dr. Trobjon Kh. Makhkamov
Department of Forestry, Tashkent State Agrarian University, 2 A., Universitet Str., Kibray district-100700, Tashkent region, Uzbekistan
E-mail: mturobzhon@mail.ru
Tel.: +998933758976

The seed is the reproductive organ of flowering plants, consisting of the seed coat, endosperm, and ovary that occurs after fertilization (Butnik *et al.*, 1991; Ezau, 1980). Seeds contain the nutrients necessary for plant growth and development. Numerous scientific sources indicate that the seeds of *V. hispanica* contain proteins, essential oils, vitamins, minerals, inactive enzymes, and unsaturated fatty acids necessary for the human body (Mazza *et al.*, 1992; Efthymiadou *et al.*, 2012; Sonnet *et al.*, 2001). The seeds are a natural source of amino acids, nutritional supplements and vegetable oils.

In the history of medicinal science of the medieval East, the merits of the great tabibs-physicians Ar-Razi, Avicenna (Ibn Sinî), the scientist-encyclopedist Beruni, Yusufi and others are enormous. When treating patients, they used medicinal plants, as well as fungi, animal (ornithological) and mineral products. Such use of plants for medicinal purposes goes back centuries (Tayjanov *et al.*, 2021).

The seeds of *V. hispanica* is a famous traditional Chinese medicine that is widely used to activate blood circulation and promote milk secretion. Previous phytochemical studies resulted in the isolation of triterpenoid saponins, cyclic peptides and flavonoids (Sang *et al.*, 2003; Jiang *et al.*, 2013).

According to the Chinese Pharmacopoeia (Committee for the Pharmacopoeia of China, 2010), seeds of *V. hispanica* are able to invigorate the circulation of blood, promote the menstrual flow and the flow of milk, and cause the subsidence of swelling, for agalactia, amenorrhea, dysmenorrhea, mastitis with swelling and pain of the breas in human medicine. Also, seeds of *V. hispanica* are used as a veterinary drug and as a feed additive to enhance the milk yield (Commission, 2015). In addition, as the data would support, seeds of *V. hispanica* have been used to treat the patients with dry eye symptoms in climacteric women (Zhang *et al*., 2012), help herpes zoster (Zhang, 2012) and help the treatment of chronic prostatitis (Huo, 2009; Shi, 2010) in clinic. Its application has also been seen inauricular acupressure, an alternative therapy (Hsieh *et al*., 2012).

The seeds of *V. hispanica* contain more than 60% starch and 15-16% protein (Price *et al*., 1987). The antifungal activity of seeds was also determined (Wong *et al*., 2017). In addition, seeds of *V. hispanica* contain triterpenoid saponins, cyclic peptides, flavonoids, carbohydrates, starches, fats, proteins, and their use in the treatment of certain diseases (Koçyiðit and Þevket, 2018; Zhou *et al*., 2016; Zhou *et al*., 2016; Erofeeva *et al*., 2019).

The number of scientific studies on invasive plants in Uzbekistan is growing day-by-day (Makhkamov, 2020; Makhkamov, 2021a, 2021b; Makhkamov and Dushaboeva, 2021). At the same time, a lot of research is being done on purposefully introduced medicinal plants (Inoyatova *et al*., 2021; Makhkamov *et al*., 2021; Polatova *et al.*, 2021; Yuldasheva *et al*., 2022). This article examines the anatomy and phytochemistry of seeds collected from the plantation of *V. hispanica*, which is grown as a medicinal crop in Uzbekistan.

2. Materials and Methods

For experiments, the seeds of *V. hispanica* were collected from plants grown in the conditions of the Tashkent oasis in 2020-2021 (in the territory of the Information Consulting Center “EXTENSION CENTER” at the Tashkent State Agrarian University).

Agroplantations of *Vaccaria hispanica* (Mill.) Rauschert.

When the seeds were ripe, they were collected and cleaned by hand. Purified seeds were dried for 10 days at room temperature (20-25°C) for quality storage. Dried seeds were stored at low temperature and humidity (15°C and 20% relative humidity) until experiments began.

2.1 Anatomic studies of seeds

The seed was fixed in 70% ethanol alcohol according to a well-known method, and was studied in cross-section in the fixed material (Trankovsky, 1979).

A magnifying glass and ruler were used to study the morphological features of *V. hispanica* seeds, and hand-cut preparations and MOTIC V1 microscope were used to study the anatomical features of the seeds.

The preparations were stained with saffron. Cell and tissue were measured using a micrometer MOB-15. Numerous measurements of several characteristics of the seed were carried out in a generally accepted way: the height of the seed coat, the thickness of the ovary, the thickness of the endosperm (Dospekhov, 1985).

Statistical analysis of the data was performed using a personal computer (MS Excel) using generally accepted methods. The microphotographs were taken using a digital camera and the mathematical analyzes were studied under a MOTIC microscope.

2.2 Phytochemistry studies of seeds

2.2.1 Determining the amount of fat

50 g of the test sample was weighed into a filter paper patron, with an error of not more than 0.01 g, a piece of fat-free cotton wool was placed on top. The cartridge, thus prepared was placed in the extractor of the Soxhlet apparatus. The flask of the Soxhlet apparatus was filled to about 2/3 of the volume with extraction gasoline, attached to the extractor, and heated in a water bath. The extraction was

continued for 8 h. Then, the patron was removed from the extractor and the solvent was distilled off from the flask into the extractor. After the extractor was filled to the upper bend of the siphon tube, the pure solvent was poured out of the extractor, which was then reattached to the Soxhlet apparatus, and the solvent remaining in the flask was distilled off. After the solvent was distilled off, the extractor was disconnected, and the flask was kept in the bath until the solvent evaporated. After the solvent had evaporated, the flask was placed in a drying cabinet and dried at 105 ± 5°C for 60 min, cooled in a desiccator, and weighed. Subsequent weighing was carried out after repeated drying for 30 min. Drying and weighing are repeated until the difference between the results of two successive weighings is not more than 0.001 g.

2.2.2 Determining the amount of total protein

The method consists in determining nitrogen according to Kjeldahl, followed by conversion to protein. The essence of the method consists in 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 test material.

From the averaged crushed homogeneous sample of the studied defatted meal of *V. hispanica* seeds, an accurate sample was weighed for analysis in a test tube, with an error of not more than 0.1%. The sample was quantitatively transferred to a Kjeldahl flask. Further, the experiments were carried out according to the guidelines (Methods of control, 2004).

2.2.3 Flavonoids

"Agilent-1200" HPLC, Column Agilent C18 5 µm, 4.6 × 250 mm. The elution was carried out in isocratic mode, a mixture of 0.1% orthophosphate acid and acetonitrile in the ratio (70:30) was used as the mobile phase. The volume flow rate of the eluent was 1.0 ml/min, the volume of the injected sample was 10 µl, length in 254 nm.

2.2.4 Isolation and identification of free amino acids

The sedimentation of proteins and peptides from the aqueous extract of the samples was carried out in centrifuge beakers. To do this, 1 ml (exact volume) of 20% TCA was added to 1 ml of the test sample. After 10 min the precipitate was separated by centrifugation at 8000 rpm for 15 min. After separating 0.1 ml of the supernatant, freeze-dried. The hydrolyzate was evaporated, the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation was repeated twice to neutralize the acid. Reaction with phenylthioisocyanate gave phenylthiocarbamyl derivatives (FTC) of amino acids according to the method of Steven and Cohen Daviel (1988). Identification of amino acid derivatives was carried out by HPLC. HPLC conditions: Agilent Technologies 1200 chromatograph with DAD detector, 75 × 4.6 mm discovery HS C18 column. Solution A: 0.14 M CH_3COONa + 0.05% TEA pH 6.4, B: CH_3CN. Flow rate 1.2 ml/min, absorbance 269 nm. Gradient % B/min: 1-6%/0-2.5 min; 6-30%/2.51-40 min; 30-60%/40.1-45 min; 60-60%/45.1-50 min; 60-0%/50.1-55 min.

3. Results

In this study, invasive plant *V. hispanica* grown in Uzbekistan as a medicinal crop was morphologically described.

Annual, plant 20-60 cm high, stem straight, usually branched in the middle. The cotyledons are oblong, hairless, 9-32 mm long and 3.5-8 mm wide. The leaves are opposite, lance shape, 2-10 cm long. The leaves are bluish green and hairless, oblong-ovate, oblong or oblong-lanceolate, almost heart-shaped at the base, acute. The leaves clasp the stem. Bracts lanceolate, very acute, membranous bordered. Lower axillary pedicels 4-6 cm long. The flowers have 5 petals and 5 sepals. Calyx oblong, 12-13 mm, with fruits up to 15 mm long, with triangular, sharp teeth, 5 times shorter than the tube. The petals are pink, 15-16 mm long, with an obovate-toothed blade, appear at the end of stems. The stamens are longer than the petals and sick out of the flower. Capsule broadly oblong, shorter than calyx.

3.1 Morphology of seeds

Seeds 1.7-2.2 mm, angular and globose-subglobose, dark brown and shiny surface covered with linear-round plates, margin crenate, surface rugose, hilum distinct (Figure 1). It was found that 1 g of seeds grown in Uzbekistan contains 150-250 seeds and 1000 seeds weigh 4.25 g. Balsevich's research has shown that 1 g of seeds contains 125-250 seeds (Balsevich, 2008).

3.2 Anatomyof seeds

On the transverse section, the seed has a rounded shape, it is covered with a seed coat on the outside (Figure1).

The peel is multilayer, protecting the ovary from various external influences and drying, premature germination. The seed coat consists of 3 layers: exotest, mesotest, and endotest (Figure 2). The seed coat contains air cavities. At the base of the skin are the ovary and endosperm (Figure 3). The endosperm is the tissue inside the seed that provides the plant with nutrients during development. The endosperm of *V. hispanica* consists of large-celled connective tissue, most of the cells of which are occupied by secondary starch.

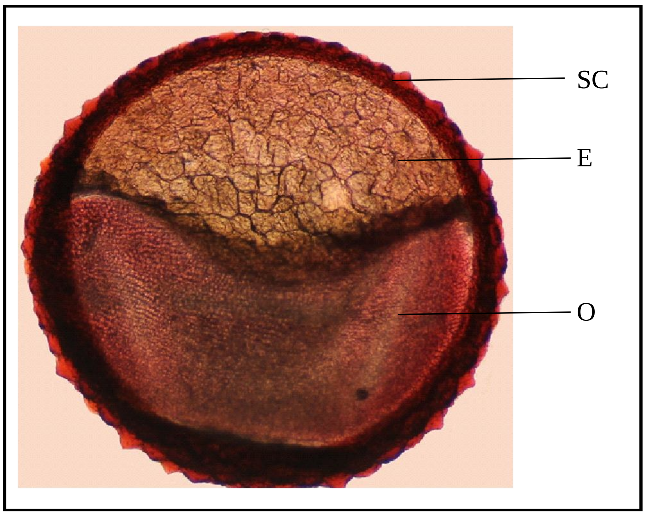

Figure 1: Internal structure of *V. hispanica* seeds: SC-seed coat, E-endosperm, O-ovary.

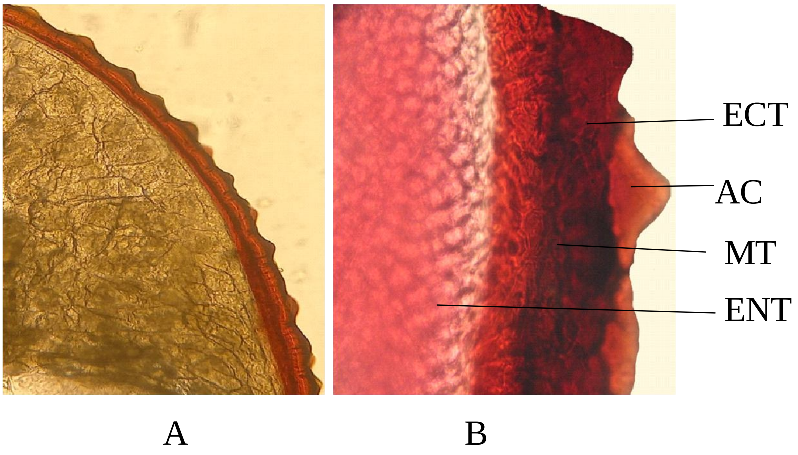

Figure 2: General view of the thick skin of the seeds of *V. hispanica*: A-endosperm side, B-overy side: AC-air cavity, ECT-exotesta, MT-mesotheta, ENT-endotesta.

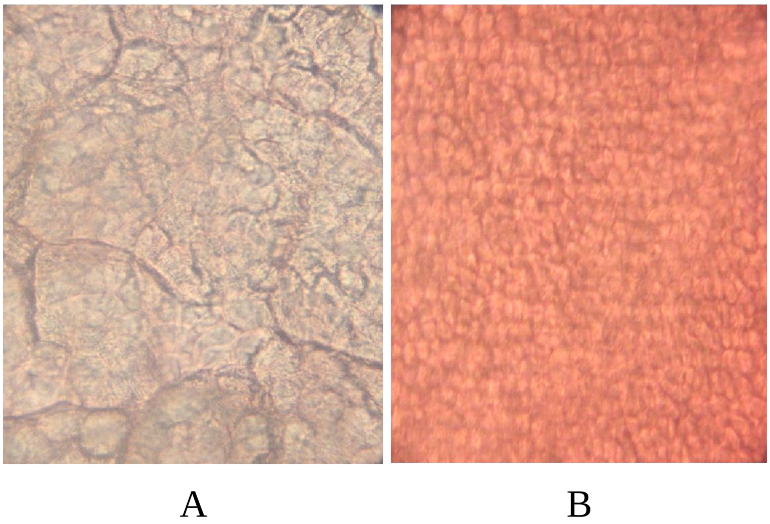

Figure 3: Microscopic view of endosperm (A) and ovary tissue (B) of *V. hispanica* seeds.

The seed coat is composed of rectangular and oval-shaped cells and is densely packed. The thickness of the seed coat is 7-10% of the total. The endosperm is composed of parenchyma tissue that is densely packed and full of chemicals (Table 1).

Table 1: Anatomical features of the seed of *V. hispanica* on cross section

No.	Features	um
1	Seed diameter	16,2 ± 0,4
2	Seed skin thickness	2,1 ± 0,1
3	Endosperm thickness	8,5 ± 0,3
4	Ovary thickness	7,7 ± 0,3

Cow cockle germs consist of an embryo (35-40% of seed weight) which nearly encircles the starchy endosperm (*e.g.*, 55-60% of seed weight).

Diagnostic signs: Densely located parenchyma cells, the presence of an ovary with 35-40% of seed volume, the presence of epithelial glands.

3.3 Phytochemistry of seeds

Seeds of *V. hispanica* which cultivated in Uzbekistan contain 4.6% fat.We did not determine the component content of the *V. hispanica* oil. There is information about this in the literature.70% of the oil contained in the seeds consists of triglycerides (predominantly 43% linoleic, 37% oleic and 13% palmitic acid) and 25% phospholipids (Balsevich, 2008).

V. hispanica seeds were determined to contain 13.6% protein (Table 2), 11.18 mg/g flavonoids (Table 3) and 18.7185 mg/g free amino acids.

Table 2: The amount of protein in *V. hispanica* seeds

Sample	Protein (%)	Nitrogen
Seed	13,58	2,53

Table 3: Composition and amount of flavonoid sin *V. hispanica* seeds

No.	Name of flavonoids	Concentration, mg/gr
1.	Dehydroquercetin	8,756
2.	Luteolin	0
3.	Rutin	2,424
4.	Quercetin	0

The study of free amino acid composition of *V. hispanica* seeds revealed the presence of 19 types of amino acids, the total amount of amino acids was 18.7185 mg/gr. of these, irreplaceable amino acids-threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine-accounted for 6.8364 mg/g or 36.5% (Table 4).

Table 4: Composition and amount of free amino acids in *V. hispanica* seeds

No.	Amino acids (AA)	Concentration, mg/gr
1.	Aspartic	0,197187
2.	Glutamic	0,240754
3.	Serine	0,396513
4.	Glycine	1,044546
5.	Asparagine	1,16679
6.	Glutamine	0,370414
7.	Cystine	0,357995
8.	Threonine*	0,734794
9.	Arginine	0,507072
10.	Alanine	0,719319
11.	Proline	0,971931
12.	Tyrosine	5,495774
13.	Valine*	2,104087
14.	Methionine*	0,255851
15.	Isoleucine*	0,143038
16.	Leucine*	0,573134
17.	Histidine	0,342172
18.	Tryptophan	0,071665
19.	Phenylalanine*	2,717124
20.	Lysine*	0,308341
	Amount	**18,7185**
	Share irreplaceableAA	**6,8364 (36,5%)**

Note: *Irreplaceable amino acids

The amino acid profile of the seeds are rich in tyrosine, phenylalanine, valine, asparagine and glycine. Tyrosine is needed to build protein molecules in all tissues, is part of enzymes, and can partially replace molecular cross-links with a lack of other substances in the body. For the formation of tyrosine, phenylalanine is necessary an essential amino acid, without which synthesis is impossible. Phenylalanine is needed for the structure of protein compounds, and all of its unused residue is converted into tyrosine.

A lack of valine leads to weight loss, growth retardation, and the development of keratoses. In adults, valine deficiency caused by a lack of B vitamins or complete proteins is accompanied by a violation of the coordination of body movements and an increase in the sensitivity of the skin to irritants. There is a negative nitrogen balance

V. hispanica seeds contain irreplaceable amino acid-lysine (0.308341 mg/g). Deficiency of lycine leads to physical and mental handicap (Papes *et al*., 2001).

As can be seen from Table 2, *V. hispanica* seeds contain 0.308341 mg/g of arginine. The antioxidant activity of these amino acids suggests a disease preventive role as exemplifies by arginine which is beneficial for preventation of cardiovascular disease (Balsubramanian *et al.*, 1980).

4. Discussion

V. hispanica considered an essential industrial weed. The presence of triterpenoid saponins contributes to their properties. Cow cockle has a lot of potential as a source of pharmaceutical products or as a medicinal plant.

As a result of our research, it was found that 1 g of *V. hispanica* seeds contain 150-250 seeds, which is practically consistent with the data of Balsevich (Balsevich, 2008), and differs only in the presence of at least 150 seeds per 1 g. In other words, seeds grown in Uzbekistan weigh less.

Similar seed morphology and granule organization have been reported for cow cockle seeds: the pericarp layer consists of a dense, compact layer of cells and can be easily removed by abrasive dehulling; starch granules in endosperm cells are polygonal in shape and range in size between 0.3-1.5 pm (Biliaderis *et al*., 1993). However, the data on the embryo (16-18% of seed weight) and endosperm (70-74% of seed weight) of cow cockle seeds presented (Biliaderis *et al.*, 1993) did not correspond to the composite mass of seeds grown in Uzbekistan. This allows us to conclude that the mass of the embryo and endosperm in the seed is not the same under different conditions.

The most valuable compounds of the *V. hispanica* are found mostly in their seeds, and about 84% of the seeds have usable compounds. Price *et al.* (1987) found that *V. hispanica* seeds contained more than 60% starch and 15-16% protein. According to Balsevich (2008) research, seeds contain 60-65% starch, 2-4% saponins, 11-14% proteins, 3-4% fat, 10-11% fiber (mostly present in the crust and some soluble matter), 9 -11% ash, 0.4-1% phenolics (with antioxidant and food preservative potency) and 0.3-1% cyclopeptidase (nutraceutical potency) were detected. The amount of protein, fat and other substances detected in the seeds as a result of our research is the same as the previous data. From this, it can be concluded that changes in the conditions of the place where *V. hispanica* is grown, do not drastically affect the amount of substances in its seeds.

5. Conclusion

V. hispanica is an important industrial weed. Large potential exists to use cow cockle as source of pharmaceutical products or as medicinal plant. *V. hispanica* is a weed that is found in the plains, hills, low and middle mountainous areas of Uzbekistan, in fields, neglected and abandoned areas, on the banks of canals, pastures, and is not used for a specific purpose. Anatomical studies of *V. hispanica* is limited and there are no reports from Tashkent/Uzbekistan. The results revealed the anatomy and phytochemistry of the seeds of *V. hispanica* grown in Uzbekistan for the first time. Also, the seed morphology was confirmed in this study.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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