

Resource assessment of bilberries (*Vaccinium myrtillus*) on Kozuf Mtn., R. N. Macedonia

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Introduction

Bilberries are one of the most used and exported natural resources in North Macedonia and the demand from pharmaceutical and food industry is constantly increasing. Collection of bilberries on Shara, Pelister, Osogovo and Kozuf mountains provides significant additional income for large number of local inhabitants.

There are three species of bilberries in R. N. Macedonia and two are found on Kozhuf Mtn: *Vaccinium myrtillus* L.-European bilberry (VM) and *Vaccinium uliginosum* L.-European bog bilberry (VU). They grow in large populations and cover vast areas. They thrive on damp acid soils, damp woods and sandy and rocky soils (Stefkov et al., 2014). Fruits (*Myrtilli fructus*) are mainly used for food and preparation of home-made juices and jams. Leaves are traditionally used for making hot or cold beverages as herbal tea. The harvest period of fruits is July - August, and May - June for the leaves, during the blooming period. Leaves are then airdried in shadow, while fruits are air dried, freezed or used immediately. Fruits are rich in anthocyanins, polyphenols recognized for their ability to provide antioxidant and anti-inflammatory protection. Berries are known for their antioxidant activity, astringent for diarrhea, nutritive value and have high reputation for their antidiabetic activity. The leaves are traditionally used as a folk remedy for their astringent, anti-inflammatory and antiseptic quality. The aim of this study is to assess the resources and economic potential of VM on Kozhuf Mtn, southern region of N. Macedonia, based on

evaluation of fruits and leaves production as well their phytochemical characterization.

The assessment is based on intensive field estimation on bilberries natural resources (annual production of fruits and leaves), chemical analyses of leaves and fruits and analysis of the importance of VM for local population.

Materials and methods

Study area

Kozhuf Mtn. is situated in the south part of North Macedonia, along the border with Greece. It covers an area of 740.48 km².

Field survey of bilberries production

Field survey was conducted during the summer in 2022, before harvesting period. Line-transect method with collecting squares was used for estimation of leaves and berries production on several localities, altitudes and habitats. The most transects were made in heathlands. The coverage of bilberries was also recorded during the field research and grouped into three categories (I < 20%; II: 20–60%; III: 60–100%).

The fruits from the squared sampling surfaces were collected in plastic containers and counted. Leaves were also collected in every second square of five transects.

Gis mapping

Estimation of the VM cover on Kozhuf Mtn. was done by remote sensing in GIS (ArcGis 9.2) based on:

- Ground truth data on VM species presence and VM coverage (collected during the field work in 2022).
- Google Earth Images – 3-band images (red, green, blue) with varying quality of resolution.
- Satellite Images – ALOS – Recent satellite images with 4 bands (red, green, blue and infrared) with 10×10 m resolution.
- Orthophoto Images – Grayscale images produced from aerial photos with resolution of 0.5 m.
- Topography vector data – Digitized 10 m topolines (isolines), and
- Landcover vector data – all of them produced by the State Authority for Geodetic Works of Macedonia.

The orthogonal surface (2-dimensional projection of land cover) was corrected for real surface of the land cover, hence including the information on the relief (slopes, depressions, etc.). The surface of the heathlands was subdivided into three categories: SI area of the heathlands with VM coverage of less than 20%; SII with coverage of 20–60% and SIII with coverage of 60–100%.

Estimation of the bilberries production

Separate average values for production (g m^{-2}) for bilberries in heathlands and pastures were calculated. Three additional average values were calculated for heathlands: P_I , P_{II} and P_{III} corresponding to the three identified coverage types. The total VM production in 2022 (BP) was calculated as a sum of VM production in the three categories of VM coverage and production in the pastures (Stefkov et al., 2014).

$$BP_{(\text{tons})} = \frac{P_{I(\text{g m}^{-2})} \cdot S_{I(\text{ha})}}{100} + \frac{P_{II(\text{g m}^{-2})} \cdot S_{II(\text{ha})}}{100} + \frac{P_{III(\text{g m}^{-2})} \cdot S_{III(\text{ha})}}{100} + \frac{P_{\text{pastures}(\text{g m}^{-2})} \cdot S_{\text{pastures}(\text{ha})}}{100}$$

Plant material, reagents and authentic samples

Plant material (fruits and leaves) for chemical analyses were collected in August 2022 and voucher specimen of *V. myrtillus*, FFUKIMV m1/22 was deposited at the Herbarium of the Institute of Pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia.

Methanol and hydrochloric acid were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany).

Evaluating the content of anthocyanins

Evaluation of the content of anthocyanins was made according Ph. Eur. 10.8 procedure for assay of anthocyanins in fresh VM fruits (*Myrtilli fructus recens*). All samples were prepared in triplicates. The spectrophotometric analyses were made on Agilent Cary 60 UV-VIS spectrophotometer.

Results and discussion

The average mass of a single fresh fruit of VM was 0.22 g. The fruit production of bilberries averaged more than 36.11 g m^{-2} . Maximal production of bilberries was reached in the altitudinal belts between 1800 and 1900 m. The production of fruits in the heathlands was multiple times higher than in the pastures.

Average production of dry leaves was more than 200 g m^{-2} . The ratio between heathlands and pastures production, in different coverage categories as well as altitudinal belts was similar as in the case of fruits.

In similar study on Osogovo Mtn. (Stefkov et al., 2014), the average fruit and leaves production was 11.7 g m^{-2} and 213.2 g m^{-2} , respectively. Consequently, the total fruits and leaves biomass was 249.11 t (from which 218.82 t in heathlands) and 1459.4 t, respectively.

The content of anthocyanins was determined 0.52%, complying to Ph.Eur. requirements (min 0.3%) and within the range reported for Lithuania (0.264-0.399% (Burdulis et al., 2007)), and 0.6% (Burdulis et al., 2009).

Conclusion

Remote sensing, ground truth data by using GIS, transect square method were successfully applied for assessment of leaves and fruits production. The total biomass on Kozhuf Mtn. in 2022 for fruits and leaves was estimated. The content of anthocyanins was 0.52%, complying to Ph.Eur. requirements.

Bilberries on Kozuf Mtn. represent significant natural resource that can provide possible economic income from fruits and leaves for the local population and beyond.

References

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