ORIGINAL ARTICLE

A C G publications

Rec. Agric. Food. Chem. 5:1 (2025) 21-33

records of agricultural and food chemistry

Digital Agriculture Approaches for Resource Assessment of Blueberry Yield on Kozuf Mountain (R. of North Macedonia) Integrating Remote Sensing, GIS and Ground-Based Research Data

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(Received January 29, 2025; Revised March 06, 2025; Accepted March 13, 2025)

Abstract: Blueberries are a valuable natural resource in North Macedonia, providing income for many people. However, underdeveloped collection, licensing and monitoring systems result in unsustainable use. This study integrates GIS and field assessments of blueberry resources on Kozuf Mountain, analyzing annual fruit and leaf production and their chemical composition. Leaf and fresh fruit production averaged 48.30 g/m² and 30.70 g/m², respectively. The total biomass of dry leaves was estimated at 5186.70 t, mainly in heathlands. The anthocyanin content in fresh blueberries, determined according to Ph. Eur. 11, was 0.52%. The total phenolic content in *Vaccinium myrtillus* leaf extract averaged 174.55 mg GAE/g DW. Sustainable blueberry use could generate 3.55 million EUR from fruit and 5.44 million EUR from leaves. This could support nearly 1400 people based on average Macedonian salaries. To strengthen the licensing and monitoring system, resource management must align with the growing demand for bilberry collection while considering the region's socioeconomic conditions. Establishing a protected area on Kozuf Mountain, promoting sustainable harvesting and raising biodiversity awareness are crucial for long-term conservation. These efforts will help preserve natural resources while ensuring continued economic benefits for local communities.

Keywords: *Vaccinium myrtillus*; biomass production; natural resources; anthocyanins.

1. Introduction

Climate change, pollution and deforestation may be the main reasons for the decline in natural resources. Blueberries (*Vaccinium myrtillus* L.) are a vital natural resource due to their well-documented nutritional and medicinal properties, with studies highlighting their positive impact on human health [1-3]. Often regarded as a "super fruit," blueberries are a rich source of bioactive compounds, including anthocyanins, polyphenols and flavonoids. The European Medicines Agency (EMA) has formally acknowledged the health benefits of bilberry fruit for acute diarrhea and inflammations of the mouth and throat, while the leaves are widely valued in medicine for their anti-inflammatory and antiseptic

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The article was published by ACG Publications
https://www.acgpubs.org/journal/records-of-agricultural-and-food-chemistry January-June 2025 EISSN:2792-0763
DOI: http://doi.org/10.25135/rfac.28.2501.3419
Available online: April 25, 2025

effects [4]. The European Pharmacopoeia 11th ed. has also included the fresh bilberry fruit, defining it as containing a minimum of 0.30% of anthocyanins, expressed as cyanidin 3-*O*-glucoside chloride per dry weight (DW) [5].

North Macedonia (hereafter referred to as Macedonia) has a vast traditional knowledge and long history of using berries, medicinal and aromatic plants (MAPs), as well as mushrooms, for food and medicines [6-7]. One significant natural resource is blueberries, whose long-standing harvesting practices provide a substantial income for many local inhabitants in the remote mountain ranges. Furthermore, the widespread presence of blueberries across many mountain ranges in Macedonia (Shar Planina, Galicica, Pelister, Karadzica, Belasica and Kozuf etc.) makes them one of the primary natural products commonly used by the local population.

Three bilberry species are reported in Macedonia, but only two grow on Kozuf Mtn.: *Vaccinium myrtillus* L. (European bilberry) and *Vaccinium uliginosum* L. (Bog bilberry). They thrive in wet, acidic soils, wet woods and sandy and rocky soils and cover large areas [7-8-9]. Besides wild-growing crops, highbush bilberry varieties (*V. corymbosum* L.) are also cultivated in Macedonia [10-11], although studies conclude that wild blueberries have significantly higher total polyphenols, flavonoids and anthocyanins content, highlighting the nutritional advantages of wild blueberries over cultivated blueberries for their health-promoting properties [12-13].

A portion of the revenue from berry harvesting is intended to support natural reserve management in Macedonia and engage local communities in protected area stewardship. However, aside from Pelister National Park, the collection, licensing, regulation and monitoring systems are underdeveloped [14]. A key issue is the lack of scientific data on natural resource availability and quality [13-14-15], which hinders the establishment of effective licensing systems. This deficiency results in two main challenges: (a) insufficient financial resources for managing protected areas due to the lack of taxation or licensing and (b) uncontrolled "wild" harvesting puts pressure on the populations, leading to unsustained collection and lower yields in the following years.

Sustainable management of wild berries requires ecological knowledge and consideration of socioeconomic and cultural contexts [16-17].

Kozuf Mountain exemplifies this issue, with proposals for a protected zone, highlighting the urgent need for clear management objectives and strategies to address increasing bilberry harvesting demands [7]. Kozuf mountain is situated in southern Macedonia, at the border with Greece, covering an area of 740.48 km^2 in Macedonia. Kozuf mountain is shared by three municipalities: Gevgelija, Demir Kapija and Kavadarci (25 settlements, ~ 60000 inhabitants, population density 35-69.9 inhabitants/km^2 (lower in rural areas), ~12300 households [18]. Its heathlands are represented by communities with blueberries (*Vaccinium myrtillus, V. uliginosum*), junipers (*Juniperus communis*) and scattered patches of *Chamaecytisus absinthoides*.

Advances in digital technologies, particularly remote sensing and GIS, have transformed the assessment of natural resources. Remote sensing, through satellite data across multiple wavelengths, enables large-scale mapping of resource distribution, while GIS provides tools for spatial analysis and integration of geographic data. Field-based methods, such as transect sampling, offer detailed, ground-level validation of remote sensing outputs. Together, these approaches enable precise assessments of blueberries' annual production, including both fruit and leaves, by combining broad-area coverage, spatial analysis and ground truth accuracy. In Southeast Europe, blueberries predominantly inhabit the pastures and heathlands and appear insignificantly in the forest. Thus, remote sensing and GIS are feasible.

Until now, the economic potential of bilberry on Kozuf Mountain has never been assessed. The main objective of this study was to perform an integrated assessment of both GIS and field assessment of blueberries' natural resources (annual production of fruits and leaves of both species) along with the chemical characterization of the fruits and leaves. By accomplishing these objectives, we aim to clarify the economic potential and importance of blueberries to the local population in Macedonia. Promoting sustainable harvesting practices and raising awareness about the importance of biodiversity conservation are essential steps towards addressing the decline in natural resources at both local and global levels.

2. Materials and Methods

2.1. Chemical Analyses

2.1.1. Plant Material

Plant material (leaves and fruits) for chemical analyses was collected on Kozuf Mtn. in August 2022. A voucher specimen of *V. myrtillus*, VM22, was deposited at the Institute of Pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University, Skopje, Macedonia. Fruits were collected in August when fully ripened. The collected leaves were air-dried in the shade and kept in plastic bags until analysis. The fruits were stored at -18°C immediately after collection until analysis.

2.1.2. Reagents and Authentic Samples

Ethanol 96% (EtOH), Ph.Eur. grade was purchased from Alkaloid AD Skopje, while methanol (MeOH) for HPLC isocratic grade and Hydrochloric acid (R) 37% (pro analysis) were purchased from Carlo Erba Reagents, Italy. Gallic acid (GA) with purity >99% for HPLC assay was purchased from Sigma−Aldrich, Germany. Folin−Ciocalteu's (FC) reagent was obtained from VWR ChemicalsBDH, France. Anhydrous sodium carbonate (Na_2CO_3) (≥99%) was purchased from Carl Roth GmbH, Germany.

2.1.3. Extraction Procedures for Bilberry Fruits and Leaves

The extraction of fresh bilberry fruits for the determination of anthocyanin content was performed according to Ph. Eur. 11 ed. Monograph [5]. For total phenolic content, the extracts were obtained following the procedure of Vrancheva et al. with slight modifications [19]. Briefly, 1g of dried ground leaves were extracted with 100 mL 40% ethanol in an ultrasonic bath (35 kHz frequency and 240 W power) for 30 minutes.

2.1.4. Determination of Anthocyanin Content in Fresh Bilberry Fruits

The anthocyanin content was determined according to the Ph. Eur. 11 ed. monograph for Fresh Bilberry Fruit (*Myrtilli fructus recens*). For such analysis, a Varian Cary 50 UV-Vis spectrophotometer was used. The samples were analyzed at 528 nm. The percentage content of anthocyanins was expressed as cyanidin 3-*O*-glucoside chloride, using the following formula:

$$Anthocyanin\,\% = \frac{A \cdot 5000}{718 \cdot m}$$

where 718 is specific absorbance of cyanidin 3-*O*-glucoside chloride, A is absorbance at 528 nm, m is mass of the substance to be examined in grams. All measurements were performed in triplicate.

2.1.5. Total phenolic Content in Dry Bilberry Leaves

The total phenolic content (TPC) of the dry leaves was obtained using the Folin-Ciocalteu (FC) assay, according to Vrancheva et al., with slight adaptations. In short, 2 mL of FC reagent (1:10 water diluted) was mixed with 0.4 mL of the sample, followed by vigorous mixing and 1-8 minutes incubation time [19-20]. After incubation, 1.6 mL 7.5% Na_2CO_3 was added. The mixture was incubated for 20 minutes at room temperature in darkness. The analyses were carried out in triplicate. Subsequently, the absorbance of the sample was recorded spectrophotometrically at 765 nm against a blank sample. The results were obtained using a calibration curve and Gallic acid as a standard in the range of 0.02-0.10 mg/mL. The results were expressed as mg gallic acid equivalent (GAE) per dry weight (DW). The equation from the calibration curve was $y = 10.272x - 0.0336$, $r^2 = 0.999$.

2.2.1. Field Survey of Blueberry Production

The field survey was conducted in the summer of 2022 before the harvesting season (July 09-18.07.2022). The line-transect method with sampling squares (0.5 m x 0.5 m) was used to estimate bilberry production at various locations, altitudes, expositions and habitats [14]. In total, 27 linear transects with 15 sampling squares each and four large plots of 16 m^2 were sampled, totaling 409 samples. They are representative of the study area and they cover the whole altitudinal range of bilberries distribution. In brief, a 75-meter-long string was stretched at various locations, usually placed from lower to higher altitudes. The sampling squares were spaced every 5 meters, delineating a survey area of 0.25 m2, which resulted in positioning 15 sampling squares per transect. Additionally, quadratic sampling points (4m x 4m) were also made (surface of 16 m^2).

Ground truth data, such as coordinates and habitat description, were collected for each transect and location. The fruits from each squared sampling surface were carefully collected in small plastic containers and they were counted. All the fruits from the quadratic samples were also collected. Leaves were collected from the linear transects and then air-dried. Thus, in August 2022, the mass of the dry leaves in each transect and the mass of the fresh fruits were measured.

During the field research, bilberry coverage was also recorded and classified into three categories (I: bilberry coverage of ≤20%; II: 20-60%; III: 60-100%).

2.2.2. GIS Mapping

A remote estimate of the blueberry heath cover on Kozuf Mountain was done by remote sensing in GIS (Geographic Information Systems) (ArcGIS 10.7) using Maximum Likelihood Classification on a composite raster from Sentinel-2 satellite imagery dated 2018-2020.

To construct the composite raster, several spectral bands from Sentinel-2 imagery were used. These included B2 (Blue), B3 (Green), B4 (Red) and B8 (Near-Infrared), all of which have a spatial resolution of 10 meters. Additionally, bands B11 and B12, which correspond to Shortwave Infrared (SWIR), were originally at a 20-meter resolution but were resampled to 10 meters for consistency.

The accuracy of the satellite image analysis was influenced by Sentinel-2 imagery quality, the Maximum Likelihood Classification method and ground truth validation. Sentinel-2 is widely recognized for its high-quality multispectral imagery and several studies have assessed its reliability in land cover classification and vegetation monitoring [18]. The Sentinel-2's multispectral bands enabled effective vegetation differentiation, while ground truth data improved classification reliability. NDVI values further refined the results. The comparison of classified outputs with field data using standard validation metrics returned an 89% accuracy, indicating an accurate estimation of blueberry health cover.

Supervised classification was carried out by assigning each pixel to a specific land cover class based on probability distributions derived from training data. Training data for blueberry heath and other land cover types (e.g., bare soil, grasslands, forests) were obtained from ground truth observations and used to define spectral signatures. These signatures were extracted from the multispectral composite raster. The classification process involved estimating the mean and covariance of the spectral values for each class and then applying the Bayesian decision rule to categorize each pixel accordingly.

After the initial classification, an accuracy assessment was performed by comparing the classified results with independent ground truth data. The classification accuracy was evaluated using the Kappa coefficient. To further refine the results, NDVI values were incorporated to enhance the discrimination of blueberry vegetation from other similar land cover types. The final classified map provided a spatially explicit estimation of blueberry heath distribution, which was then divided into three categories: S_I area of the heathlands with bilberry coverage of less than 20%; S_{II} with coverage of 20–60% and S_{III} with coverage of 60–100%.

2.2.3. Estimation of Bilberry Fruit Production

The average production (g/m^2) of blueberries was determined according to the established methodology described by Stefkov et al. [14]. Briefly, the production on heathlands and pastures was calculated separately. Heathlands production was divided into three groups (P_I, P_{II} and P_{III}) following the three coverage categories - S_I, S_{II}, S_{III}, respectively. The plotting method yielded information on bilberry production per square meter, taking into account altitude, coverage and exposure, while GIS mapping offered insights into the spatial arrangement of grasslands on Kozuf Mountain, including their altitude-based distribution and, notably, the extent of grassland coverage. The results for the average production of fruits and leaves are presented in g/m^2 with calculated standard errors (SE). To determine the total bilberry production (BP, in tons) in 2022 on Kozuf Mtn., the sum of the production in the three categories was calculated:

$$BP\ (tons) = \frac{P_I\left(\frac{g}{m^2}\right)\cdot S_I(ha)}{100} + \frac{P_{II}\left(\frac{g}{m^2}\right)\cdot S_{II}(ha)}{100} + \frac{P_{III}\left(\frac{g}{m^2}\right)\cdot S_{III}(ha)}{100} + \frac{P_{pastures}\left(\frac{g}{m^2}\right)\cdot S_{pastures}(ha)}{100}$$

Where P is production in g/m^2 and S is surface coverage in hectares (ha).

3. Results and Discussion

3.1. Bilberry Fruits and Leaves Production Assessment on Kozuf Mtn.

The assessment of blueberry production encompassed several steps: obtaining data from the field survey, GIS mapping and estimation of the blueberry production. Figure 1 presents the study area encompassed by the GIS analysis.

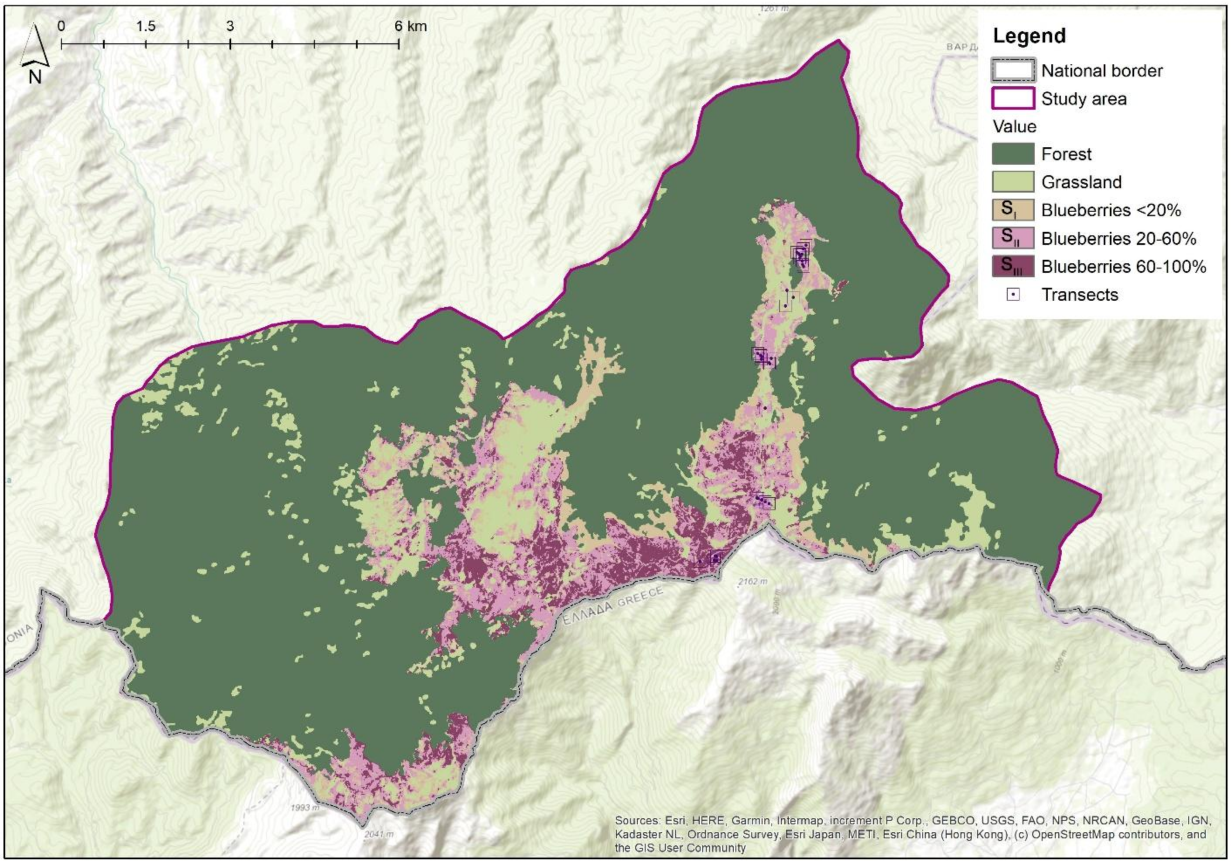

Figure 1. The study area encompassed the GIS analysis and transects. The bilberry coverage categories are illustrated with different colors (S_I – yellow, S_{II} – pink, S_{III} – purple)

The field study conducted in July 2022 assessed bilberry (*Vaccinium myrtillus*) production on Kozuf Mountain, covering 31 sampling points, including three quadratic sampling plots and 28 linear transects, within an altitudinal range of 1650-2100 m. The majority of transects were in heathlands, often accompanied by *Bruckenthalia spiculifolia* and *Juniperus communis*. The ground truth data on transects from the field survey, together with the number of collected berries per transect, are given in Table 1. Leaves were collected from 15 transects, after which they were air-dried and their mass was measured. The average mass of a single fresh bilberry (0.22 g) was determined for the purposes of bilberry production assessment.

Table 1. Ground truth data from the field survey of bilberry production conducted in July 2022.

Transect	*Start coordinates*	*End coordinates*	*Number of berries per transect*	*Mass of dry leaves per transect (g)*
Day 1 (11.07.2022)				
1	41.207669°N 22.220814°E	41.207910°N 22.219958°E	17	2.87
2	41.205150°N 22.220450°E	41.205546°N 22.219649°E	240	49.32
3	41.2065185°N 22.2221164°E	41.206894°N 22.222925°E	112	7.10
4	41.197149°N 22.215258°E	41.196816°N 22.216063°E	117	2.79
5	41.197618°N 22.214482°E	41.197235°N 22.215190°E	1167	110.07
6	41.1973272°N 22.2150552°E	41.1971185°N 22.2158679°E	843	18.85
Day 2 (13.07.2022)				
7	41.213127°N 22.223828°E	41.213120°N 22.223375°E	209	41.24
8	41.213302°N 22.223660°E	41.213867°N 22.224121°E	189	38.44
9	41.214306°N 22.224408°E	41.214905°N 22.224885°E	557	21.27
10	41.2134785°N 22.2234877°E	41.2138152°N 22.2227377°E	175	52.32
11	41.213409°N 22.224166°E	41.213889°N 22.223656°E	0	23.95
12	41.2148331°N 22.2250005°E	41.2152820°N 22.2244543°E	1633	130.28
13	41.2137483°N 22.2230247°E	41.2142762°N 22.2223146°E	879	95.40
14	41.2128353°N 22.2236327°E	41.21180°N 22.22421°E	352	76.12
15	41.21180°N 22.22421°E	41.211019°N 22.224335°E	333	49.72
Day 3 (14.07.2022)				
16	41.1959225°N 22.2169693°E	41.195398°N 22.216383°E	398	
17	41.174404°N 22.214069°E	41.174191°N 22.214848°E	2677	
18	41.196787°N 22.217261°E	41.197180°N 22.216528°E	1056	
19	41.1961263°N 22.2168254°E	41.1966889°N 22.2163199°E	563	
20	41.1744845°N 22.2138935°E	41.1748568°N 22.2132859°E	2203	

Cont'd…			
Cont'd…			
21	41.1738117°N 22.2155936°E	41.1735316°N 22.2163510°E	488
22	41.174188°N 22.214867°E	41.173819°N 22.215584°E	392
23	41.173523°N 22.216382°E	41.173225°N 22.217077°E	989
Day 4 (15.07.2022)			
24	41.1652125°N 22.2048175°E	41.164636°N 22.204795°E	426
25	41.164627°N 22.204608°E	41.163884°N 22.204693°E	143
26	41.164393°N 22.201561°E	41.1639401°N 22.2020515°E	577
27	41.1651549°N 22.2050941°E	41.1645615°N 22.2049758°E	235
Day 5 (16.07.2022)			
28	41.2114491°N 22.2244446°E		1550
29	41.212374°N 22.224163°E		4219
30	41.1967154°N 22.2152094°E		1678

The estimated total fresh fruit biomass for 2022 on Kozuf Mountain was 886.42 t (Table 2). Heathlands accounted for 883.66 t, which represents the amount of production exploited on Kozuf Mountain. The remaining 2.76 t was produced in pastures, but due to the low yield and high collection effort required, this portion is not utilized. Bilberry fruit (fresh) production was estimated at 30.70 g/m² on average, with fruit production in heathlands reaching 51.00 g/m², which exceeds the production in pastures by over 250-fold (Table 3). Although fruit production in pastures is significantly lower, it should not be entirely disregarded given the extensive pasture areas on Kozuf Mountain. The total biomass of dry leaves was estimated at 5186.70 t, with 4897.85 t from heathlands and 288.85 t from pastures. The average dry leaf yield was 48.30 g/m² (Table 2), with the production in heathlands recorded at 76.00 g/m², notably higher than the 13.40 g/m² observed in pastures. It should be noted that bilberry production follows natural fluctuations, occurring in (2)3-5-year cycles, which are synchronized across populations according to the mast hypothesis and are influenced by environmental factors [8]. The majority of herbivores that consume bilberries experience peak population levels one year after bilberry seed production peaks [8].

Table 2. Fruit and leaves biomass (tons) in forest, pastures and the three categories of heathlands.

Habitats	Surface (km^2)	Surface (%)	Leaves Biomass (t)	Fruits biomass (t)
Pastures	11.06	38.00	288.85	2.76
I (<20%)	4.86	16.70	1111.78	124.39
II (20-40%)	8.19	28.10	982.44	310.81
III (>60%)	4.99	17.10	2803.63	448.46
Total	29.10	100.00	5186.70	886.42

Table 3. Fresh fruit and dry leaves production (g/m^2±SE) and number of fruits per m^2 in forest, pastures and the three categories of heathlands.

Data	Forest	Pastures	I (<20%)	II (20-40%)	III (>60%)	Average
Total number of fruits per m^2	0	1.13±0.18	116.25±0.41	172.44±0.42	407.92±2.19	140.40±0.19
Total fruits mass (g/m^2)	0	0.25±0.08	25.58±0.19	37.94±0.20	89.74±1.03	34.42±0.09
Total mass of dry leaves (g/m^2)	0	13.40±12.17	52.40±33.30	45.60±43.61	130.28±29.70	48.30±38.70

In 2008, a resource assessment of blueberries on Osogovo Mountain estimated fresh fruit production at 249.11 t (218.82 t in heathlands) [14]. Compared to Kozuf Mountain's 886.42 t (883.66 t in heathlands) of bilberry production in 2022, the yields on Osogovo Mountain were significantly lower. Similarly, dry leaf production on Kozuf Mountain in 2022 was 5186.7 t (4897.85 t in heathlands), which was nearly 3.5 times higher than the 1459.4 t recorded on Osogovo Mountain. Fresh fruit and dry leaf production on Kozuf Mountain were measured at 30.7 g/m^2 and 48.3 g/m^2, respectively, surpassing Osogovo Mountain's production rates of 11.70 g/m^2 for fruits and 21.32 g/m^2 for leaves. Additionally, bilberry density on Kozuf Mountain was recorded at 140.4 fruits/m^2, slightly exceeding the 137.4 fruits/m^2 recorded on Osogovo Mountain [14].

A study conducted in Finland estimated the total bilberry production to be approximately 208 million kg per year, a significantly larger yield compared to the Kozuf and Osogovo Mountains [21]. However, it is essential to consider that the Finnish study assessed a vastly larger area of productive forest land, spanning across the country, whereas Kozuf and Osogovo represent much smaller mountainous territories. The difference in total yield is, therefore, not only a result of ecological or climatic factors but also largely due to the scale of the study. Nevertheless, these findings highlight the substantial contribution of Finnish forests to bilberry production, reinforcing the importance of large-scale assessments in evaluating national berry resources. Furthermore, while direct bilberry yield estimations were not provided, Bohlin et al. (2021) presented an alternative approach to assessing bilberry production through the integration of airborne laser scanning (ALS) data with field inventory data [22]. Their study demonstrated that ALS-derived metrics, particularly those related to canopy cover and light penetration, can serve as effective predictors of bilberry yield. This methodology offers a valuable tool for large-scale yield estimation without requiring extensive ground-based sampling. Applying such remote sensing techniques in areas like Kozuf and Osogovo could enhance yield assessments, providing more precise spatial distribution data and supporting sustainable management strategies.

These findings indicate that Kozuf Mountain holds significant potential for sustainable bilberry harvesting, provided that proper management practices are established. Given the fluctuations in bilberry production and the importance of maintaining ecosystem balance, a structured harvesting approach with controlled exploitation, licensing systems and research expansion is necessary [8,23]. Further assessments of natural product resources in Macedonia's mountainous regions remain scarce, primarily due to the need for extensive research teams. However, considering the economic and ecological importance of bilberry harvesting, future studies and sustainable management strategies should be prioritized to maximize the potential of this natural resource [24-25-26]. As bilberry production fluctuates from season to season, depending on climatic and other factors, for more accurate monitoring, it is necessary to collect data annually across seasons [8].

Climate change is expected to significantly impact bilberry populations on Kozuf Mountain by altering temperature, precipitation patterns and seasonal dynamics. Rising temperatures and prolonged dry periods could reduce bilberry yields, while extreme weather events such as late frosts and heavy rainfall may disrupt flowering and fruiting cycles. Additionally, changes in climate could lead to shifts in vegetation composition, competition with other plant species and altered pollinator and herbivore activity [27]. To maintain sustainable harvesting, adaptive management strategies, such as enhanced monitoring through remote sensing, GIS-based tracking and conservation measures, should be implemented. Looking ahead, several future scenarios must be considered. Bilberry populations may decline due to reduced soil moisture or shift to higher altitudes, affecting accessibility and local economies. Alternatively, with proper management, bilberry yields could stabilize through sustainable harvesting, afforestation and soil moisture retention techniques [15]. In extreme cases, climate change may lead to the replacement of bilberry-dominated heathlands with more drought-resistant species. To mitigate these risks, long-term monitoring of bilberry growth trends, phenological shifts and soil conditions will be crucial for developing predictive models and conservation strategies.

3.2. Economic Insights

To maintain sustainability, it is recommended to collect a maximum of 80% of the fruit and 30% of the leaves from a single location [7,14]. Based on these recommendations, sustainable harvesting would yield approximately 709 t of fresh bilberry fruits and 1556 t of dry leaves. With market prices in Macedonia of 5 EUR/kg for fresh bilberries and 3.56 EUR/kg for dry leaves, the potential annual revenue could reach 3.55 million EUR and 5.54 million EUR, respectively, totaling approximately 9.09 million EUR. The economy in European countries regarding bilberries leads to significantly higher prices, with fresh fruit prices ranging from 10 EUR/kg in France to 13 EUR/kg in Germany, presenting a potential for higher revenue for bilberry collectors [28].

Bilberry collection has been an important income source for residents in rural and economically less developed regions of Macedonia. In the early 1970s, approximately 500 t of bilberry fruits were collected annually in Macedonia [14]. The traditional method of bilberry harvesting involves using wood or metal combs, with a single collector capable of gathering 20-30 kg in an 8-hour work period. With an established buyout price of up to 5 EUR/kg for fresh fruits, the economic potential of bilberry harvesting remains significant. Considering that the average annual gross income per person in Macedonia in 2023 was 6612 EUR (State Statistical Office), the estimated total revenue of 9.09 million EUR from bilberry harvesting could sustain the yearly earnings of approximately 1374 people [29].

Many local inhabitants rely on bilberry production and the quality of its fruits and leaves [6-7]. Given that the total fresh fruits and dry leaves biomass on Kozuf Mountain in 2022 was estimated to be 886.42 t and 5186.70 t, respectively, it greatly supports the plan to create a protected area on Kozuf Mountain [7]. Efficiently managing natural resources requires a blend of ecological understanding of the species and communities, combined with socioeconomic and cultural factors. Implementing a system of controlled and sustainable bilberry harvesting, including the licensing of collectors, could provide an opportunity to enhance the local economy and employment strategies. Additionally, a structured approach to bilberry collection could establish new benchmarks in the region, such as organic harvesting standards and improved welfare for collectors. Another strategy for sustainable resource management in the region includes cultivating bilberry cultivars [15-17].

3.3. Chemical Composition of Bilberry Fruits and Leaves

For research purposes, the leaves collected from the linear transects were sorted into four categories, representing four different locations: Vm-L1, Vm-L2, Vm-L3 and Vm-L4 (Table 4). Briefly, location 1 had the lowest bilberry coverage compared with location 2, which had the highest. *Juniperus communis* was more prevalent in locations 2 and 4, which were also largely represented by *Pinus sylvestris*. Location 3 represents one of the first locations from where the seasonal bilberry collecting starts because the blueberries here ripen earlier. A large portion of location 3 was destroyed by fire in the past (transects conducted at higher altitudes) and the bilberry coverage was very small, but in lower altitudes on this location, blueberries were dominant.

Fresh bilberry fruits' anthocyanin content was determined to be 0.52% (SD = 0.0279, RSD = 5.35), which complies with the Ph. Eur. 11 ed. monograph requirements (min 0.30%). These results fall within the reported range of 0.26-0.40% and 0.60% for blueberries collected from several locations in Lithuania [30-31]. The intrinsic value of Kozuf Mtn. bilberry berries and leaves are enhanced by their high content of industrially important phenolic compounds. Anthocyanins constitute a major portion of total phenolic compounds (35-74%) in studies on cultivated blueberries in Macedonia [11]. The total phenolics in blueberries comprised 23-56% hydroxycinnamic acid derivatives, with chlorogenic acid being the main hydroxycinnamic acid present.

Table 4. The collected leaves were divided in four different locations (Vm-L1-4).

Location	Abbreviation	Transects	Altitudinal range (m)
1	Vm-L1	1 − 3	1682 − 1738
2	Vm-L2	4 − 6	1719 − 1739
3	Vm-L3	7 − 13	1650 − 1698
4	Vm-L4	14 − 15	1710 − 1750

In a previous study on bilberry fruits collected from Shar Planina Mtn. (Macedonia), 16 different anthocyanins were identified in *V. myrtillus* and 13 anthocyanins in *V. uliginosum*, representing the most dominant compounds of the total phenolic content (72% for bilberry, 39% for bog bilberry) [10]. The anthocyanin content in V. myrtillus was determined to be 507 ± 1 mg/100 g fresh weight, whereas in *V. uliginosum*, it was 152 ± 1 mg/100 g fresh weight. In comparison, a study on four different varieties of cultivated *V. corymbosum* in Macedonia identified 17 anthocyanins in total [10]. The highest anthocyanin content was found in the Duke variety, evaluated at 83.64 ± 3.16 mg/100 g fresh weight, which is significantly lower than wild blueberries.

Chemical composition assessment of leaves included TPC analysis of the dry leaf samples from the linear transects. They were sorted into four categories, representing four different locations: Vm-L1, Vm-L2, Vm-L3, Vm-L4, presented in Table 2. The dry leaves sample Vm-L3 showed the greatest TPC (184.62 ± 2.55 mg GAE/g DW). Conversely, the lowest phenolic content was measured in sample Vm-L2 (162.19 ± 1.08 mg GAE/g DW). The leaves from VM on Kozuf Mtn. had an average TPC of 174.55 ± 2.66 mg GAE/g DW (Table 5).

Table 5. Total phenolic content in bilberry leaves collected from four different locations (Vm-L1-L4).

Sample	Total Phenolic Content, mg GAE/g DW	RSD
Vm-L1	176.32 ± 3.50	1.98
Vm-L2	162.19 ± 1.08	0.67
Vm-L3	184.62 ± 2.55	1.38
Vm-L4	175.10 ± 3.53	2.02
AVG	**174.55 ± 2.66**	**1.51**

The findings related to the chemical composition and total phenolic content (TPC) of bilberry leaves align with previously documented information. The TPC of bilberry leaves from Bulgaria, collected in May 2019, ranged from 33.72 ± 0.22 to 66.37 ± 0.31 mg GAE/g DW [19]. These values are lower than the TPC measured in this study (average 174.55 ± 2.66 mg GAE/g DW). This discrepancy is reasonable, considering that TPC levels increase during summer months in response to higher light intensity and exposure [32-33]. In bilberry leaves from Osogovo Mtn., a total of 13 phenolic components were identified, with hyperoside, chlorogenic acid and its isomer 5-caffeoylquinic acid being the major representatives, contributing 13.09 mg/g dry extract, 0.35 mg/g dry extract and 11.89 mg/g dry extract, respectively [14]. These findings were similar to those derived from bilberry leaves from Shar Planina

Mtn., where caffeoylquinic acid was the predominant phenolic acid derivative, contributing 57% in V. myrtillus and 64% in *V. uliginosum* [10].

Bilberry leaves are also recognized as a significant reservoir of chlorogenic acid [35]. Beyond chlorogenic acid, extracts from the leaves of Vaccinium species serve as a rich natural repository for various phenolic compounds, including flavonoids and proanthocyanidins. The bilberry leaves, berries and stems constitute a phenolic biological resource containing over 116 different chemical compounds. Leaves are particularly rich in kaempferol, quercetin, isorhamnetin and laricitrin, often found as free aglycones. They also contain an abundance of glycosides, including quercetin, isorhamnetin, myricetin and syringetin [31].

As indicated in the literature, phytochemical analyses confirm that blueberries are a valuable resource of biological phytocomponents. However, sourcing from natural habitats can pose standardization challenges due to potential variations in the quantity of biological compounds [36].

Acknowledgments

We would like to thank the students from the Scientific Research Association (SRA) from the Faculty of Pharmacy – Skopje for participating in the field studies. This research wouldn’t have been possible without your true passion and dedication to science. This research was supported by the Young Ecologists Support Program “d-r Ljupčo Melovski” organized by the Macedonian Ecological Society – MES.

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