

## Effect of Drying Methods on Phytochemical Compounds, Color Attributes, and Antioxidant Activity of *Ecballium elaterium* (L.) Fruits

Nasrin Hossein zadeh<sup>1</sup>, Hassanali Naghdi Badi<sup>2,3</sup>, Sepideh Kalateh Jari<sup>1,\*</sup>, Ali Mehrafarin<sup>3</sup> and Sakineh Saeidi Sar<sup>4</sup>

<sup>1</sup> Department of Agricultural Science and Engineering, SR.C., Islamic Azad University, Tehran, Iran

<sup>2</sup> Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahed University, Tehran, Iran

<sup>3</sup> Medicinal Plants Research Center, Shahed University, Tehran, Iran

<sup>4</sup> Department of Agricultural Science, Technical and Vocational University (TVU), Tehran, Iran

Corresponding author: Email: kalatehjari@yahoo.com, S.kalatehjari@gmail.com

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### ABSTRACT

This study investigated the effects of various drying methods on the physicochemical and phytochemical properties of *Ecballium elaterium* L. fruits. A completely randomized design with three replications was employed to evaluate sun, shade, microwave (200, 600, 1000 W), oven (35, 45, 55°C), infrared (0.2, 0.3, 0.4 W), and vacuum oven (35, 45, 55°C) drying methods. Results demonstrated significant ( $p \leq 0.01$ ) impacts on all measured parameters except total cucurbitacin (TCu). Vacuum oven drying at 55°C optimally preserved quality parameters, showing the highest antioxidant activity (AA), total phenolic content (TPC), and total flavonoid content (TFC), along with superior color retention (L, a, b\*). Conversely, sun and infrared drying caused the greatest degradation of total free amino acids (TFAAs) and total soluble proteins (TSP). Microwave and infrared drying exhibited power-dependent negative effects, with higher power levels significantly reducing TPC, TFC, and color parameters. Shade drying showed intermediate preservation of phytochemicals, while oven drying at 55°C maintained moderate quality. The study revealed strong correlations between drying kinetics and phytochemical preservation, with slower drying methods generally demonstrating better retention of bioactive compounds. These findings provide critical insights for selecting optimal drying techniques to maintain the nutritional and medicinal value of *E. elaterium* fruits during post-harvest processing, with microwave 200, oven and vacuum oven drying at 45°C emerging as the most effective method for quality preservation.

**Keywords:** Alkaloids, Antioxidant activity, Free amino acids, Microwave, Vacuum

### INTRODUCTION

The squirting cucumber, scientifically known as *Ecballium elaterium* (L.) A. Rich is a plant from the Cucurbitaceae family renowned for its medicinal properties. Studies have shown that its fruit extract possesses a variety of therapeutic properties, including the treatment of conditions such as cancer, sinusitis, fever, constipation, liver cirrhosis, rheumatic disease, high blood pressure, dropsy, and jaundice [2, 15]. Moreover, the juice derived from the fruit of the squirting cucumber is a significant source of carbohydrates, proteins, lipids, tannins, gum, minerals, and triterpenoids, particularly cucurbitacins [12]. Changes in enzyme activity and phytochemical compounds, such as cucurbitacins, in the squirting cucumber can have potential implications for its medicinal properties. They may impact the therapeutic efficacy of the plant extract in treating various diseases. Understanding these changes and their effects on the biological activities of the squirting cucumber is crucial to harnessing its full potential in medicinal applications [1, 12]. The squirting cucumber is indigenous to the Mediterranean region, particularly in the countries of southern Europe, North Africa, and the Eastern Mediterranean, and can also be found in parts of the Middle East. This plant is commonly cultivated in climates with warm, sunny, and well-drained soil. It is often grown for ornamental purposes due to the distinctive nature of its fruit. The cultivation of *E. elaterium* typically involves minimal care and is commonly initiated from seeds. The growing season for this plant typically commences in spring and continues through the summer months. Beyond its ornamental appeal, various parts of the *E. elaterium* plant have been utilized in traditional medicine for diverse purposes. However, it is imperative to recognize that the plant contains toxic compounds and should be handled with caution [21]. In summary, *E. elaterium* is a captivating plant with historical and scientific importance, and its cultivation can provide an engaging and enlightening experience for gardeners and botanists alike.

Drying, an age-old method utilized for the preservation of herbs, serves the purpose of dehydration to inhibit the proliferation of yeasts, enzymes, and microorganisms, ultimately prolonging the shelf life of the herbs. Some researchers have spotlighted the significance of this technique [27, 31]. Furthermore, the process of drying diminishes the bulk and mass of the herbs, resulting in cost savings associated with packaging, storage, and transportation, as emphasized by Aghdam et al. [3] and Chakraborty et al. [10].

Today, various techniques are employed in the dehydration of medicinal herbs, including natural methods such as shade and sun drying, as well as artificial techniques including hot air, oven, vacuum, and microwave radiation [24, 28]. Using natural methods for drying plant materials is considered a primary approach due to cost-effectiveness, which had disadvantages such as inconsistent achievement of quality standards and handling large quantities of plant material [9]. The application of microwave radiation with its rapid diffusion in plant material has a significant benefit in terms of shorter drying time and also preserving the color and active components of dried plants [35]. Vacuum drying is a gentle alternative technique to dry sensitive or easily oxidized products without increasing the temperature [30, 26]. However, during the drying process, enzymatic activities may alter the constitution of active components in the medicinal herbs [24] and

the phytochemical compounds may change [30]. Of course, the influence of the technique employed for drying these compounds is contingent upon various factors, including plant species, duration, and temperature of the drying procedure [7].

According to research, methods such as oven dehydrating at 30 and 40°C, or sun and shade drying did not have a noteworthy effect on the composition and essential oil (EO) content of *Rosa damascena* [4]. In contrast, Mashkani et al. reported that different drying techniques had significant impacts on the EO content and composition of *Thymus daenensis* [24]. Therefore, assessing various drying techniques is important, as these methods can have a significant influence on the chemical composition and biological activity of the plants. The squirting cucumber is a medicinal plant with various phytochemical compounds and enzymatic activities that contribute to its therapeutic properties. The drying process of *E. elaterium* can significantly alter the composition of phytochemicals and enzyme activities, thereby impacting its pharmaceutical potential. This study aims to investigate the effects of different drying techniques on the quality indices of *E. elaterium*, focusing on phytochemical compounds and enzyme activities. Understanding these effects can help the pharmaceutical, nutraceutical, and herbal medicine industries optimize the drying process to preserve or enhance the beneficial properties of this plant.

## MATERIALS AND METHODS

This study was conducted at the Iranian Institute of Medicinal Plants (IMP) of the Academic Center for Education, Culture and Research (ACECR). The fruit samples were obtained from Meshginshahr (38°40'N and 47°66'E) in Ardabil province, Iran. Fruits were harvested at the physiological maturity stage when they were still green, before turning to a yellow hue. This precaution was taken because ripe yellow fruits, under the pressure of accumulated fluid, are vulnerable to rupturing, leading to the dispersion of seeds and liquid content.

### Sample Preparation

Fruits with the same appearance in terms of shape, size, color, and absence of physical damage were collected at 8-9 in the morning and dried by exposure to either sun, shade, oven, vacuum (35, 45, and 55°C), microwave (200, 600, and 1000 W) or infrared (0.2, 0.3 and 0.4 W). The study examined 14 drying methods and samples were compared with fresh fruit (Table 1).

**Table 1** Abbreviations and full names of treatments

Row	Treatments abbreviations	Treatments name
1	Sun	Sun drying
2	Shade	Shade drying
3	O45	Oven drying at 45°C
4	O55	Oven drying at 55°C
5	O65	Oven drying at 65°C
6	V45	Vacuum drying at 45°C
7	V55	Vacuum drying at 55°C
8	V65	Vacuum drying at 65°C
9	I 0.2	Infrared drying at 0.2 W
10	I 0.3	Infrared drying at 0.3 W
11	I 0.4	Infrared drying at 0.4 W
12	M200	Microwave drying at 200 W
13	M600	Microwave drying at 600 W
14	M1000	Microwave drying at 1000 W

### Drying Methods

#### Natural Drying Methods

The sun drying method involves placing fruit samples on a pristine and pale fabric exposed to direct sunlight. Conversely, the shade drying method entails the distribution of samples in a space without direct sunlight, while maintaining the room temperature consistent (25°C ± 1) [24].

#### Artificial Drying Methods

Following the techniques outlined by Mashkani *et al.* [24], a thin layer of the samples was dispersed evenly across the trays and subjected to the oven, microwave, vacuum, or infrared drying, individually.

#### Determining the Moisture Content

The water content of the fruit was determined using the following equation:

$$\% \text{ Moisture} = (mf - mw / mf) \times 100 \quad (1)$$

Where: *mf* is the weight of fresh fruit and *mw* is the sample weight after dehydration.

#### Color Measurement

The CIELAB color scale (*L\**, *a\**, *b\**) is a three-dimensional color space that is widely used in the food industry to measure and quantify color. The *L\** axis represents lightness, with values ranging from 0 (black) to 100 (white). The *a\** axis represents the red-green color component, with positive values indicating redness and negative values indicating greenness. The *b\** axis represents the yellow-blue color component, with positive values indicating yellowness and negative values indicating blueness [43]. In the present research, the characteristics of color were evaluated with the Laboratory color meter model YS6060 (3nh, China).

## Biochemical Analysis

The chemical materials utilized in this research were procured from the Merck company, in Germany. Fruits underwent a drying and grinding process to produce homogenized powder samples. The drying step facilitated moisture control and enhanced the overall grinding ability. Moreover, grinding improved the drying efficiency [17].

### Total Soluble Protein (TSP)

For TSP determination, 500 mg homogenized samples were mixed with 5 ml potassium phosphate buffer (10 mM, pH 7.0, 4% PVP), centrifuged (HB705, 16700 g, 4°C, 26 min), and the supernatant's absorbance at 595 nm (Visible/UV-45 Lambda) was measured after adding Bradford reagent, using BSA standard curve [8].

### Total Free Amino Acids (TFAAs)

For TFAAs, 0.5 g of fruits were homogenized in 80% ethanol, centrifuged (17600 g), mixed with ninhydrin reagent (citrate buffer/ninhydrin/glycerol=2:5:12), heated (98°C, 16 min), and absorbance at 570 nm was measured against glycine standard [41].

### Cucurbitacin B

Cucurbitacin B analysis involved methanol extraction (65°C, 30 min) followed by HPLC (Hitachi, C18-Lichrospher-100 column, 125×4.6 mm, 5 µm) with mobile phase (methanol/acetonitrile/0.28% acetic acid=5:10:35, 1 ml/min flow rate), detected at 360 nm (injection volume: 20 µl) (Figure 1).

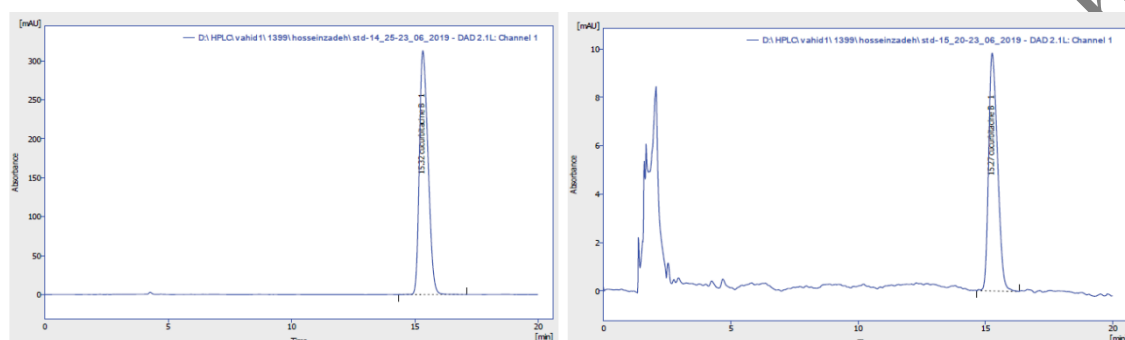


Fig. 1 Chromatograms of cucurbitacin B standard (left) and *Ecballium elaterium* extract (right)

### Total Alkaloid

The total alkaloid content was determined spectrophotometrically by dissolving 5.5 ml methanolic extract in 1 ml concentrated HCl, followed by filtration and triple washing with chloroform. The aqueous phase was neutralized (pH 7 with 0.1 N NaOH), mixed with bromocresol green reagent and phosphate buffer (pH 7.4), and the chloroform phase was collected. Absorbance was measured at 470 nm (Visible/UV-45 Lambda), with alkaloid percentage calculated as:  $TA (\%) = (W/Y) \times 100$ , where W = alkaloid weight and Y = sample weight [38].

### Insoluble Tannin

200 mg dried fruit was homogenized in 10 ml aqueous acetone (7:3 v/v), stirred (30 min, 4°C), and centrifuged (20,000 g, 20 min, 4°C). The supernatant (tannin-free) was analyzed for total phenolics at 280 nm after PVPP sedimentation [23].

### Total Flavonoid Content (TFC)

Extraction of 0.4 g powder with 80% methanol (12 hr, 25°C) was followed by reaction with  $\text{NaNO}_2$ ,  $\text{AlCl}_3$ , and NaOH. Absorbance at 510 nm was measured, with TFC expressed as mg quercetin equivalent/g sample [19].

### Total Phenolic Content (TPC)

Folin-Ciocalteu assay was performed on 80% methanol extracts (12 hr, 25°C) mixed with Folin reagent and  $\text{Na}_2\text{CO}_3$ , incubated (45°C, 15 min), and measured at 765 nm. Results were expressed as mg gallic acid equivalent/g [19].

### Antioxidant Activity (AA)

DPPH assay involved mixing methanolic extracts (80%, 12 hr) with 0.1 mM DPPH, incubating (30 min, dark), and measuring absorbance at 515 nm. The scavenging percentage was calculated as:  $\% \text{ Scavenging} = (A_0 - A_1)/A_0 \times 100$ , where  $A_0$  = control and  $A_1$  = sample absorbance [19].

### Statistical Analysis

Data were analyzed using SAS software and the generalized linear model (GLM) procedure. The differences among the drying methods were compared at a 5% confidence interval using the least significant difference (LSD) tests. Additionally, R software was employed for the purpose of principal component analysis (PCA) and plots.

## RESULTS AND DISCUSSION

### Moisture Loss and Drying Time

The analysis of variance results showed that different drying methods had a significant effect (at the 1% probability level) on the moisture content, drying time, and colorimetric indices ( $L^*$ ,  $a^*$ , and  $b^*$ ) of the samples (Table 2).

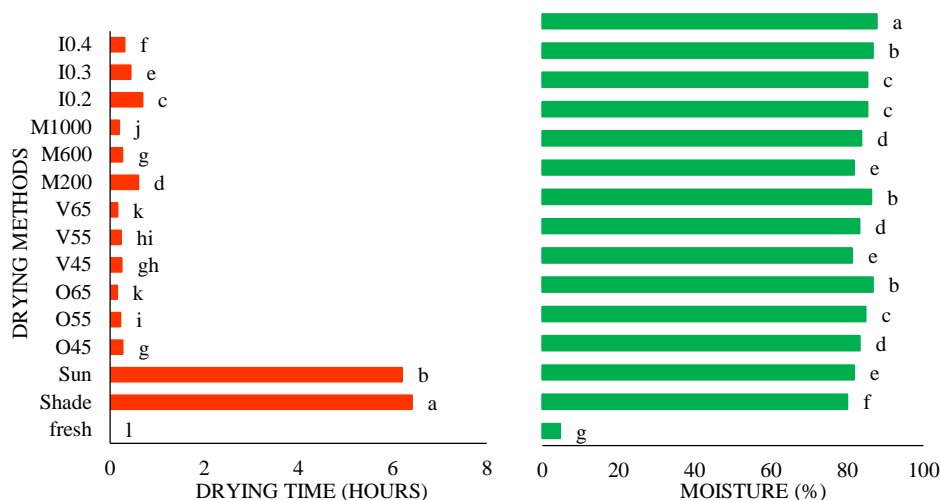
The longest drying times were observed in natural methods (shade drying followed by sun drying) at 641 and 620 minutes, respectively. Increasing power and temperature in artificial drying methods accelerated the drying process so that samples dried faster in the oven at 55°C. These methods also resulted in significantly higher moisture loss (87.8% for M600) compared to natural drying (80%-81.8%). The O65 method proved to be the fastest, completing the drying process in just 0.14 hours (Figure 2).

**Table 2** Analysis of variance for the effects of different drying methods on the drying time, moisture, and color parameters of *Ecballium elaterium* (L.) fruits

Sources	DF	Mean Squares				
		Moisture	Drying time	<i>L</i> *: lightness	<i>a</i> *: redness	<i>b</i> *: yellowness.
Drying method	14	8.15 **	5539840 **	546.6 **	179.6 **	204.9 **
Error	30	0.10	3.37	1.32	0.37	0.22
CV (%)	-	6.5	0.4	2.8	10.3	3.0

\*\* : significant at the  $p < .01$  level; ns: non-significant

Since natural drying methods required considerably longer processing times (several days) compared to artificial drying methods which were completed within a short timeframe (less than one hour), the drying time values for shade and sun drying methods were displayed in the graphs with a scaling factor of  $\times 10^3$  to enable comparison of the different time scales (Figure 2).



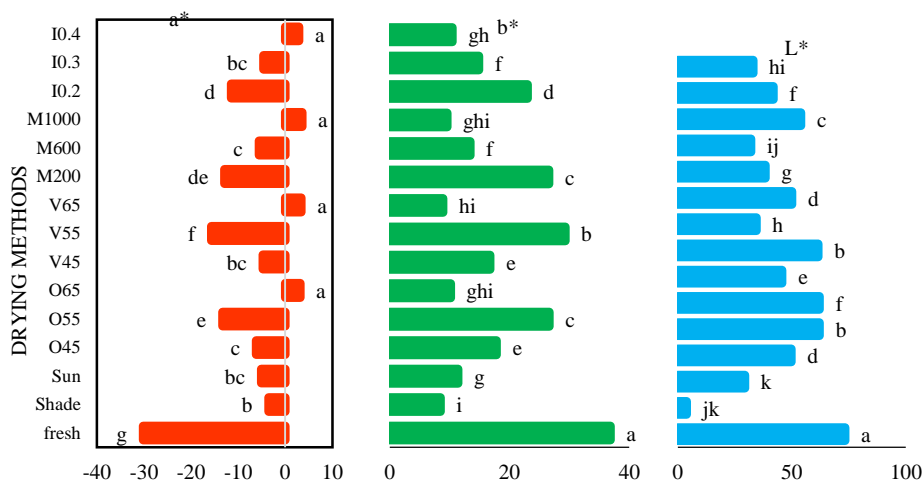
**Fig. 2** The effects of different drying methods on the drying time and moisture loss of *Ecballium elaterium* (L.) Fruits. Means followed by similar letters in each column are not significantly different at the  $p < .05$  level based on the LSD test. The full name of treatments is presented in Table 1.

\*\* means for sun and shade in drying time is means  $\times 10^3$ .

Drying reduces packaging, storage, and transportation costs while reducing product volume and weight. Low moisture content products can be stored at room temperature. Drying time is influenced by plant moisture level and ambient temperature, with lower moisture content leading to faster drying and higher temperatures enhancing moisture evaporation. Research shows that microwave drying reduces the time needed to achieve the desired moisture content and yields superior results compared to oven drying [29]. Microwave radiation is quick and efficient for plant samples, with the intensity and drying time influenced by oven and microwave power and infrared radiation [16]. Based on a study, higher oven temperatures, power, microwave and infrared radiation led to a decrease in drying time and an increase in drying speed and intensity for *Agastache* leaves [20]. This is consistent with previous findings for *Dracocephalum kotschyi* [42] and rosemary plants [37], as moisture reduction is influenced by water movement from internal layers to the plant's surface.

### Color Parameters

Based on the results, different drying techniques significantly impacted color parameters, with a notable increase in the  $a^*$  (2) parameter and a decline in both  $L^*$  (22) and  $b^*$  (Figure 3).



**Fig. 3** The effects of different drying methods on the color parameters of *Ecballium elaterium* (L.) fruits. L\*: lightness; b\*: from blue to yellow; and a\*: from green to red. Means followed by similar letters in each column are not significantly different at the  $p < 0.05$  level based on the LSD test. The full name of treatments is presented in Table 1.

The L\*, a\*, and b\* color spaces are commonly used for food color measurement due to their uniform distribution and close approximation to human perception. Color observation is crucial for consumers to accept or reject dried herbs, and it helps identify defects that may compromise food quality.

Colorimetric analysis (L\*, a\*, b\*) revealed that in natural drying methods (shade and sun), the highest reduction in L\* parameter was observed (62.5% and 68.5%, respectively) compared to fresh plants. Among artificial methods, vacuum ovens, microwaves, and infrared at 55°C showed the most significant increase in color parameters, while the smallest decreases in L\* were recorded in O55 (23%) and V55 (17%) drying. Notably, the M1000 method also caused a considerable reduction (61.2%) in L\* value. It should be noted that all drying methods led to L\* reduction, with the most pronounced decrease observed in sun drying (Figure 3). However, vegetables and fruits turn brown due to enzymatic and non-enzymatic browning responses. Low temperature conditions prevent enzymatic browning, ensuring stable color parameters [30].

The present study revealed that different drying methods significantly affected the color parameters L\* (lightness), a\* (red-green component), and b\* (yellow-blue component). Among natural drying methods, sun drying caused the most pronounced changes in all color parameters, indicating the most severe color degradation.

For artificial drying methods, the highest b\* values (indicating yellowness) were observed in vacuum drying at 55°C (with the following order: vacuum 55°C > oven 55°C > microwave 200W > infrared 0.2W). The a\* parameter showed a significant shift toward positive values (redness) in high-power and high-temperature methods.

Vacuum and oven drying at 55°C best preserved color quality by maintaining L\* values and preventing extreme changes in a\* and b\* parameters. The microwave method at 200W and the infrared method at 0.2W also effectively controlled color changes and prevented color degradation.

These results demonstrate that the selection of the drying method and optimization of its parameters play a crucial role in preserving the color characteristics of the final product (Figure 3) [5, 32, 34].

### Total Soluble Protein (TSP) and Total Free Amino Acids (TFAAs)

The ANOVA results revealed that drying methods significantly affected ( $p < 0.01$ ) total soluble proteins (TSP), total free amino acids (TFAA), total alkaloid (TA), insoluble tannin (ITa), total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity (AA) (Table 3).

**Table 3** Analysis of variance for the effects of different drying methods on the biochemical characteristics of *Ecballium elaterium* (L.) fruits

Sources	DF	Mean Squares							
		TSP	TFAA	TCu	TA	ITa	TFC	TPC	AA
Drying method	14	5.08 **	13.90 **	0.0043 ns	1331 **	0.44 **	4.51 **	69.48 **	41.61 **
Error	30	0.27	0.11	0.0029	107.47	0.29	0.11	0.76	0.28
CV (%)	-	1.56	2.44	6.51	1.45	3.96	2.34	1.25	1.08

\*\*: significant at the  $p < .01$  level of probability; ns: non-significant

Df: degrees of freedom; TSP: total soluble protein; TFAA: total free amino acids; TCu: total cucurbitacin; TA: total alkaloids; ITa: insoluble tannin; TFC: total flavonoid content; TPC: total phenol content; and AA: antioxidant activity.

Among natural methods, shade drying showed superior performance in preserving total soluble proteins (TSP) with the highest content (35.9 mg/gDW). For artificial drying methods, microwave drying exhibited the best preservation of total free amino acids (TFAA) with the smallest decrease (15.4-16.2 mg/gDW), while infrared drying caused the most significant losses in both TFAA (10.4-10.9 mg/gDW) and TSP (31.5-31.9 mg/gDW). Intermediate TSP preservation was observed in oven and vacuum oven drying, ranking second after shade drying (Table 4).

**Table 4** Comparison of the effectiveness of various drying methods on the biochemical properties of *Ecballium elaterium* (L.) fruits.

Drying	TSP	TFAA	TA	ITa	TFC	TPC	AA (%)
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	(mg/gDW)	(mg/gDW)	(mg/gDW)	(mg/gDW)	(mg/gDW)	(mg/gDW)	(mg/gDW)
Fresh	35.9 ± 0.15 a	17 ± 0.01 a	762 ± 0.34 a	14.5 ± 0.06 a	16.2 ± 0.16 a	76.6 ± 0.32 a	53.7 ± 0.03 a
Shade	35.4 ± 0.09 a	14.3 ± 0.01 f	759 ± 0.81 a	14.1 ± 0.14 abc	14.2 ± 0.01 cd	70.9 ± 0.30 d	44.8 ± 0.01 e
Sun	32.3 ± 0.01 d e	12.4 ± 0.01 h	699 ± 23.1 d	13.8 ± 0.08 abc	13.6 ± 0.58 e	69.3 ± 0.20 ef	47.9 ± 0.01 d
O45	34.0 ± 0.02 b	14.8 ± 0.01 ef	705 ± 0.38 d	13.6 ± 0.04 bc	14.3 ± 0.20 c	69.8 ± 0.26 def	51.8 ± 0.06 b
O55	34.1 ± 0.02 b	14.9 ± 0.02 de	712 ± 0.28 cd	13.4 ± 0.01 bc	15.3 ± 0.03 b	73.5 ± 0.35 bc	52.2 ± 0.06 b
O65	33.7 ± 0.01 bc	14.7 ± 0.01 ef	708 ± 0.14 d	13.8 ± 0.02 abc	13.3 ± 0.08 e	63.5 ± 1.49 h	51.9 ± 0.70 b
V45	33.9 ± 0.05 b	12.0 ± 0.01 g	704 ± 0.60 d	13.9 ± 0.02 abc	14.3 ± 0.15 c	72.4 ± 0.42 c	53.2 ± 0.04 a
V55	34.1 ± 0.01 b	12.2 ± 0.01 h	709 ± 0.24 d	13.6 ± 0.01 bc	15.7 ± 0.03 ab	74.7 ± 0.24 b	53.6 ± 0.03 a
V65	33.7 ± 0.02 bc	12.1 ± 0.01 g	702 ± 0.15 d	14.3 ± 0.12 ab	13.6 ± 0.10 e	68.4 ± 0.22 f	52.0 ± 0.71 b
I0.2	31.9 ± 0.02 ef	10.9 ± 0.02 h	703 ± 0.31 d	13.3 ± 0.01 c	13.3 ± 0.01 e	68.6 ± 0.19 f	44.3 ± 0.02 e
I0.3	31.8 ± 0.01 ef	10.7 ± 0.02 h	701 ± 0.48 d	13.4 ± 0.15 bc	12.6 ± 0.15 f	64.8 ± 0.31 gh	44.0 ± 0.03 ef
I0.4	31.5 ± 0.02 f	10.4 ± 0.01 h	697 ± 0.21 d	13.4 ± 0.01 bc	11.4 ± 0.21 g	58.9 ± 0.54 i	43.3 ± 0.04 f
M1000	32.4 ± 1.15 de	15.6 ± 0.34 c	728 ± 0.44 bc	13.7 ± 1.19 abc	13.7 ± 0.12 de	65.0 ± 0.41 g	48.7 ± 0.05 cd
M200	32.9 ± 0.01 cd	16.2 ± 0.030 b	733 ± 0.23 b	13.2 ± 0.01 c	15.1 ± 0.02 b	74.8 ± 0.32 b	48.9 ± 0.065 c
M600	32.6 ± 0.02 de	15.4 ± 0.57 cd	731 ± 0.31 b	13.3 ± 0.02 c	14.5 ± 0.14 c	70.1 ± 0.13 de	49.1 ± 0.04 c

Means followed by similar letter in each column are not significantly different at the  $p < .05$  level based on LSD test.

Means ± standard error.

TSP: total soluble protein; TFAA: total free amino acids; TCu: total cucurbitacin; TA: total alkaloids; ITa: insoluble tannin; TFC: total flavonoid content; TPC: total phenol content; and AA: antioxidant activity.

The full name of treatments is presented in Table 1.

The differences in the effects of drying methods on TFAA and TSP levels can be attributed to the differences in heating mechanisms used. Microwave drying uses internal dielectric heating, causing rapid temperature increases, while infrared drying uses radiant heat transfer, affecting degradation rates [33]. The drying time and temperature profiles of different drying methods can influence the degradation of TFAA molecules. Microwave driers offer faster drying, promote energy efficiency, and preserve heat-sensitive compounds, but may accelerate degradation due to shorter exposure to damaging conditions [22]. High-temperature drying can decompose secondary metabolites, destroy the plasma membrane and cell wall [40], and cause the loss of phytochemical compounds [18]. TSP content significantly decreases with drying, with a range of 1.4 to 12.2% compared to fresh plants. The shade drying method had the lowest reduction, followed by the oven and vacuum oven methods. The amount of TFAAs and TSP decreases with increasing temperature and drying power, consistent with studies on *Thymus daenensis* and *Dracocephalum kotschyi* [36]. The highest and lowest reductions of TFAAs were obtained in infrared and microwave drying, respectively. The TSP content is higher when dehydrated in oven, vacuum an oven, and shade (Table 4).

According to Table 4, microwave drying at 200 W showed the lowest loss of TFAAs compared to other methods, possibly due to rapid and localized heating within the sample. However, TFAA content was highest at this power setting, possibly due to the rapid release or leaching of TFAAs from the tissue [22, 33]. Future studies could investigate the potential migration or leakage of TFAAs during microwave drying. Infrared drying at 0.4 W resulted in the greatest reduction in TFAAs (Table 4), likely due to gradual heating by infrared radiation. Further research could explore the influence of different infrared wavelengths on TFAA retention [22, 31]. The diverse impacts of drying methods on TSP and TFAA levels can be attributed to a complex interplay of factors, including chemical structure, stability, localization, interaction with other cellular components [13], and specific drying techniques [25]. Differences in retention may not solely stem from individual factors but from their combined influence [6].

#### Total Cucurbitacin (TCu), Total Alkaloids (TA) and Insoluble Tannin (ITa)

The study found no significant effect of drying methods on total cucurbitacin B (TCu) content, while total alkaloid (TA) and Insoluble tannin (ITa) were significantly affected. Natural drying methods showed better preservation - shade drying maintained the highest levels of TA (0.4% decrease), TCu, and ITa. Among artificial methods, vacuum oven drying at 55°C showed relatively good preservation (7.5% TA decrease), followed by oven drying at 55°C (7.0%). Microwave drying at 200W caused moderate reductions (4.1% TA decrease), while infrared drying showed power-dependent effects: lower power (0.2W) caused less TA reduction (703) than higher power (0.4W, 697). The most severe reductions occurred in sun drying (8.3% TA decrease, among natural methods) and infrared drying at 0.4W (lowest TA and ITa values overall) (Table 4).

Variations in the amount of secondary metabolites through the drying procedure, depend on temperature, time, tissue type, and drying method [24]. Additionally, during the drying process, water from plant tissues can evaporate, resulting in the removal of some metabolites [11]. Finally, the amounts of TA and ITa of the fruits, dried in the shade are significantly higher than the other methods. The drying process significantly altered the profile of secondary metabolites in the fruits, particularly TA, and ITa. Compared to fresh samples, all drying methods led to reductions in these compounds, albeit to varying degrees. Shade drying emerged as the gentlest approach, minimizing losses of TA, and ITa; which suggests that slower drying at ambient temperatures preserves the integrity of these bioactive components. Microwave and oven drying followed closely in terms of preserving TA, potentially benefiting from their rapid drying time which limits exposure to potentially degrading conditions.

#### Total Pphenol Content (TPC), Total Flavonoid Content (TFC) and Antioxidant Activity (AA)

The study revealed the differential effects of drying methods on bioactive compounds. Among natural drying methods, all showed significant reductions in total phenolic content (TPC, 2.3-23%), total flavonoid content (TFC, 3.2-29.6%), and antioxidant activity (AA, 0.09-19.4%). For artificial drying methods, vacuum oven drying at 55°C demonstrated superior preservation, maintaining the highest TPC (71.8 mg/g) and TFC levels, closely followed by microwave drying at 200W (70.0 mg/g TPC). Notably, infrared drying caused the most substantial decreases in both TPC and TFC among all methods. The fresh fruit control showed the highest values (76.6 mg/g TPC), with

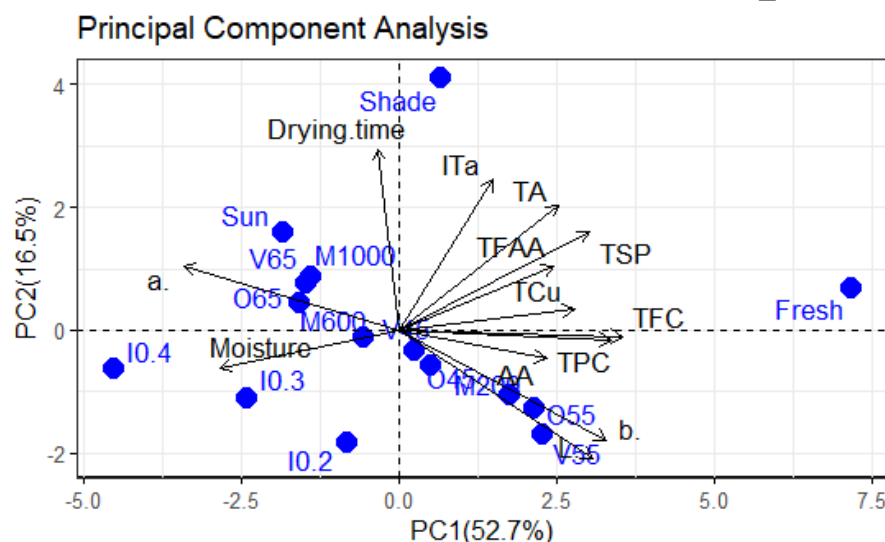


vacuum oven and microwave-dried fruits exhibiting the best retention of bioactive compounds among processed samples (Figure 4, Table 4).

TPC and TFC are important antioxidant parameters, but their reductions highlight the trade-off between preservation and convenience. Vacuum oven and microwave drying are the most promising methods for preserving these parameters, with moderate reductions and superior results. Rapid drying with a controlled temperature or gentle drying at a moderate temperature can minimize bioactive losses. Conversely, infrared drying proved detrimental to TPC and TFC, implying that its intense internal heating might promote accelerated degradation of these sensitive compounds [13, 22]. The study highlights the importance of selecting the right drying method for squirting cucumber fruits, considering factors like drying time and energy efficiency. The study found that the antioxidant activity (AA) was lower in dried fruits than in the control, except for vacuum oven-dried fruits, which had the highest TPC [14]. This suggests that certain drying techniques, like vacuum oven drying at 45°C, might effectively preserve specific contributors to AA by minimizing thermal degradation or promoting favorable interactions between different bioactive compounds. The observed discrepancies in AA across drying methods warrant further investigation. The highest activity was observed in vacuum oven and oven drying at 45 and 55°C, while the significantly lower AA associated with infrared drying at 0.4 W likely arises from intense internal heating [25]. Understanding the specific mechanisms underlying these differential effects could provide valuable insights for optimizing drying strategies for maximum antioxidant preservation. The study also found that increasing the infrared and microwave power during the drying process decreased the radical scavenging parameter of squirting cucumber fruits [15]. The squirting cucumber fruit's essential compounds and antioxidants have attracted attention due to their chemical preventive effects against diseases related to oxidative stress [39].

### Principal Component Analysis (PCA)

The study analyzed the connections between analyzed characteristics and the co-linearity of drying techniques with measured characteristics using principal component analysis (PCA). The first two components explained 70.68% of the total variation, with the primary principal component (PC1) accounting for 52.7% and showing affirmative associations with all characteristics. The second principal component (PC2) accounted for 16.5% of the total variance and showed a negative correlation with  $L^*$ ,  $b^*$ , and TCu content but a strong positive correlation with ITa and TSP contents (Figure 4 and Table 5).



**Fig. 4** Principal component analysis (PCA) results of *Ecballium elaterium* (L.) fruits measured characteristics under various drying methods.  $a^*$ : green to red;  $b^*$ : blue to yellow;  $L^*$ : lightness; ITa: Insoluble tannin; TA: Total alkaloid; TSP: Total soluble protein; TFAA: Total free amino acids; AA: Antioxidant Activity; TFC: Total flavonoid content; TPC: Total phenolic content; TCu: Total cucurbitacin.

The full name of treatments is presented in Table 1.

The full name of abbreviations is presented in Table 5.

All traits except tannin content and drying time are correlated with Principal Component 1 (PC1). Traits with positive loadings (e.g., freshness) are associated with the right side of the biplot, while those with negative loadings (e.g., moisture,  $b^*$ ) align with the left side and exhibit greater dispersion. In contrast, tannin content and drying time are correlated with Principal Component 2 (PC2). These traits show a negative correlation with vacuum- and infrared-based drying methods but a positive correlation with natural drying conditions (shade and sunlight) (Figure 4 and Table 5).

The drying techniques were classified into four groups: microwave, oven, vacuum oven and natural by sun or shade. The microwave, oven, and vacuum oven were characterized by elevated levels of TFC and TPC, as well as  $L^*$  and  $b^*$  color parameters. The shade drying process was distinguished by higher levels of TSP, TA, and TCu. The fourth group, consisting of sun, microwave, oven, and vacuum oven, exhibited elevated levels of  $a^*$  and ITa contents (Figure 4). The study found that various drying methods effectively differentiated dried squirting cucumber fruits based on secondary metabolites. The PCA biplot analysis indicated that drying with a 200 W microwave, oven, and vacuum oven at 45°C was the optimal method for *E. elaterium* fruits, as it preserved a greater number of secondary metabolites and prevented browning.

**Table 5** Principal component loadings for the characters measured under different drying methods

Character's name	Abbreviation	PC1	PC2
Total phenol content	TPC	0.87	0.05
Total flavonoid content	TFC	0.93	0.03
Antioxidant activity	AA	0.60	-0.10
Total free amino acids	TFAA	0.67	0.27
Total alkaloids	TA	0.68	0.53
Total soluble protein	TSP	0.79	0.41
Insoluble tannin	ITa	0.38	0.67
Total cucurbitacin	TCu	0.73	0.06
Lightness	$L^*$	0.79	-0.55
Redness	$a^*$	-0.88	0.28
Yellowness	$b^*$	0.85	-0.48
Moisture		-0.74	-0.15
Drying time		-0.09	0.75
Eigenvalue		6.98	2.22
Percent of variation		53.6	17.08
Cumulative percentage of variation		53.6	70.68

## CONCLUSIONS

In this work, we studied how different methods of drying and related factors affected the color and number of secondary metabolites in dried *E. elaterium* fruits. Results indicated that different methods of drying had significantly impacted the color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) of the dried fruits. Particularly, it was shown that vacuum oven drying, medium temperature oven drying (45°C), and low power microwave (200 W) were the most successful techniques for keeping the color of the dried fruits. Especially for *E. elaterium*, oven and vacuum oven drying at 45°C proved to be the most successful methods for maintaining the secondary metabolites and avoiding fruit browning.

Our findings also showed while the contents of TPC, TFC, TSP,  $a^*$ , and  $b^*$  increased with drying time, the content of particular secondary metabolites, such as AA, TFAA, TA, ITa, and  $L^*$ , increased with drying speed. These results highlight the complex effects of the drying methods and related parameters on the quantitative and qualitative properties of the active ingredient in *E. elaterium*. Consequently, our research emphasizes how crucial method selection is to achieve the best possible performance of the active ingredients. It also, highlights the important influence that methods of drying and their particular conditions have on the amount and quality of secondary metabolites in *E. elaterium*. These results indicate the importance of choosing a suitable drying method to achieve the desirable function of the active component, which is essential for applications in a variety of industries, such as food, cosmetics, and pharmaceuticals.

## AUTHOR CONTRIBUTIONS

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## CONFLICT OF INTEREST

The authors affirm that they have no competing financial interests to disclose. Moreover, they declare no involvement in any activities that could potentially compromise the objectivity of this paper.

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