Journal of Food Composition and Analysis 151 (2026) 108989

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Berberine from *Berberis aristata*: How stable isotope analysis enables the discrimination between natural and synthetic origins

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ARTICLE INFO

Keywords:
Berberine
Berberis aristata
Stable isotope
Synthetic
Natural

ABSTRACT

Berberine is a benzylisoquinoline alkaloid traditionally sourced from plants such as *Berberis aristata* and *Coptis chinensis*, widely used for its therapeutic properties. However, the high cost of natural extraction has led to the increasing use of cheaper synthetic berberine derived from petrochemical sources, raising concerns over product authenticity. This study investigates the stable isotope composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$) of natural berberine extracted from *Berberis aristata* and synthetic samples produced through unknown chemical routes, likely involving catechol-based starting materials, using isotope ratio mass spectrometry (IRMS). Among the tested isotopic parameters, $\delta^{13}C$ proved to be the most reliable for origin discrimination, with natural berberine showing significantly lower values (–33 ‰ to –32 ‰) than synthetic products (–30.6 ‰ to –29.7 ‰). The $\delta^{2}H$ provided complementary differentiation, although variability in synthetic pathways can produce partial overlap with natural products. Conversely, $\delta^{15}N$ and $\delta^{18}O$ displayed limited discriminating power due to overlapping ranges. A market survey of low-cost commercial berberine supplements labelled as natural revealed that most samples matched isotopic values typical of synthetic berberine, indicating widespread undeclared substitution. These findings highlight the importance of stable isotope analysis as a robust tool for authenticity verification and quality control of botanical ingredients in consumer products.

1. Introduction

Berberine is a naturally occurring quaternary ammonium salt that belongs to the group of benzylisoquinoline alkaloids (Fig. 1). Berberidaceae (*Berberis vulgaris*, *Berberis aquifolium*, *Berberis aristata*, and *Iododendron amurense*), Coptidaceae (*Coptis chinensis* and *Coptis japonica*) and Ranunculaceae (*Hydrastis canadensis*) plants showed relatively high concentration of berberine (see Table 2 in (Neag et al., 2018)). Depending on the plant species, berberine can be found primarily in the roots, rhizomes, bark, or petioles, with concentrations varying significantly among these anatomical parts.

Berberine is increasingly used today due to its beneficial properties. Traditionally, berberine has been employed in Chinese medicine, mainly for the treatment of gastrointestinal disorders such as diarrhoea (Yu et al., 2020) and dysentery (Chang, 1959). The effectiveness of the alkaloid is based on multiple mechanisms: inhibition of intestinal fluid secretion, modulation of gastrointestinal motility, enhancement of the integrity of the intestinal barrier, and broad–spectrum antimicrobial action (Xia et al., 2014).

Lower lipid levels and enhanced insulin sensitivity are the most extensively studied effects of berberine in contemporary clinical research, supported by numerous randomized clinical trials (Imenshahidi and Hosseinzadeh, 2019). Notably, the hypolipidemic mechanism of berberine differs from that of statins: while statins inhibit HMG-CoA reductase, berberine lowers cholesterol primarily by upregulating LDL receptor expression, thereby enhancing LDL clearance through an independent pathway (Kong et al., 2004). In addition, berberine may further contribute to cholesterol reduction by decreasing intestinal absorption and promoting excretion (Wang et al., 2014). Berberine has also shown potential in improving insulin sensitivity, likely through modulation of insulin signalling and glucose metabolism (Wang et al., 2011). Moreover, emerging evidence supports the potential

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https://doi.org/10.1016/j.jfca.2026.108989
Received 20 November 2025; Received in revised form 21 January 2026; Accepted 9 February 2026
Available online 11 February 2026

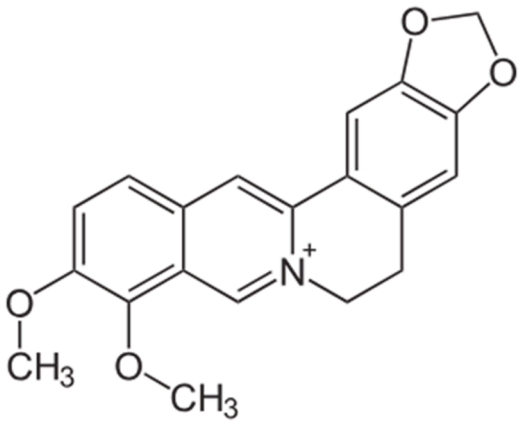

Fig. 1. Berberine chemical structure.

use of berberine in managing conditions such as polycystic ovary syndrome (PCOS), with multiple clinical studies suggesting its efficacy (Imenshahidi and Hosseinzadeh, 2019). However, for other indications, including cancer, hypertension, and stroke, the current body of clinical evidence remains limited, often restricted to isolated or preliminary studies and comprehensive clinical trials are necessary to establish berberine therapeutic relevance in these areas (Imenshahidi and Hosseinzadeh, 2019).

Not all the commercially available berberine products are extracted from plants. In recent years, several methods for the production of synthetic berberine have been reported in the scientific literature (Mori−Quiroz et al., 2018; Tajiri et al., 2021; Zhou and Tong, 2016). Nevertheless, the most commonly employed industrial synthesis route utilizes catechol, a readily accessible precursor (Shenyang University of Chemical Technology 沈阳化工大学, 2019). As a result, synthetic berberine is widely available on the market and offered at a significantly lower price than the naturally extracted compound, typically 25−30 % cheaper than the natural one.

This price discrepancy introduces the risk of economically motivated adulteration. Unscrupulous manufacturers may substitute natural berberine with its synthetic counterpart in dietary supplements, potentially compromising both consumer trust and the validity of clinical trials evaluating natural formulations. Consequently, it is mandatory to develop robust analytical methodologies able to distinguish between natural and synthetic sources of berberine, to ensure product authenticity and integrity.

Stable isotope ratio analysis has emerged as a powerful tool in this context. The isotopic composition of bioelements, specifically carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$), and hydrogen ($^{2}H/^{1}H$), can provide information about the origin and the biosynthetic pathway of a compound, even when its natural form is chemically identical to the synthetic one. A comprehensive overview of the application of stable isotope ratio analysis for the discrimination between natural and synthetic molecules, with particular emphasis on compound-specific approaches, has been recently provided by Perini et al. (2024).

The carbon isotope ratio ($\delta^{13}C$) is influenced by the photosynthetic pathway of the source plant. According to O'Leary et al., C4 plants typically exhibit $\delta^{13}C$ values between –14 ‰ and –12 ‰, while C3 plants range from –30 ‰ to –23 ‰ (O'Leary, 1988; Perini et al., 2024). Fossil−derived precursors, which are frequently used in chemical syntheses, often show $\delta^{13}C$ values from –35 ‰ to –19 ‰ (Degens, 1969). This isotopic parameter was already effective in distinguishing biosynthetic lovastatin from monacolin K derived from red yeast rice fermentation (Perini et al., 2017), as well as in differentiating synthetic L−theanine from that naturally extracted from *Camellia sinensis* (Perini et al., 2021).

Synthetic processes may introduce isotopic fractionation effects that are reflected in the hydrogen isotope ratio ($\delta^{2}H$). For instance, synthetic vanillin shows significantly higher $\delta^{2}H$ values (average +63 ‰) compared to its natural counterpart (average –32 ‰) (Perini et al., 2019) and a high $\delta^{2}H$ value is a marker for synthetic curcuminoids, distinguishing them from turmeric−derived natural extracts (Perini et al., 2023).

In this study, for the first time, the isotopic ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), and hydrogen ($\delta^{2}H$) in berberine, either derived from *Berberis aristata* or by chemical synthesis, were systematically investigated. The analysis was performed using an Isotope Ratio Mass Spectrometer (IRMS) coupled with an Elemental Analyzer (EA) and a Pyrolizer (P). The objective of this study was to develop consistent stable isotopic criteria capable of effectively discriminating between naturally derived and synthetically produced compounds.

2. Materials and methods

2.1. Sampling

Twenty−two samples of authentic berberine HCl powder (>95 %) extracted from *Berberis aristata* dried roots (BN−1 to BN−22, Table 1) coming either from Southeast Asia or China and thirteen samples declared as 100 % synthetic berberine powder (BS−1 to BS−13, Table 1) were provided by VIVATIS S.p.A., Milan, Italy. As declared by the company, plant material was subjected to acidic aqueous–ethanolic extraction, followed by filtration and concentration under reduced pressure. Crude extracts were then purified by pH adjustment and crystallization, a widely used industrial approach to obtain berberine hydrochloride with purity ≥ 97 %. The resulting material was dried, finely ground, and homogenized prior to isotopic analysis.

The pathway that had been followed to produce the synthetic products was not available. The samples were produced in different years between 2019 and 2025. Finally, nine commercial berberine powder samples (>95 %) declared as from natural products were purchased from the market (BC−1 to BC−9, Table 2).

2.2. Stable isotope analysis of berberine

Stable isotope ratios of hydrogen, carbon, nitrogen, and oxygen were analysed in berberine powder. This method is rapid and largely automated, taking less than 10 min per analysis.

The $\delta^{13}C$ and $\delta^{15}N$ were determined in a single run (sample weight 0.8 mg loaded in tin capsules) via isotope ratio mass spectrometry (IRMS) (Isoprime Ltd., UK), following total combustion in an elemental analyser (VARIO CUBE, Elementar Analysensysteme GmbH, Germany). Similarly, $\delta^{2}H$ and $\delta^{18}O$ were measured in one run (sample weight 0.25 mg loaded in silver capsules) using a Finnigan DELTA XP IRMS (Thermo Scientific, Bremen, Germany), equipped with a high temperature pyrolizer (Finnigan TC/EA, Thermo Scientific, Bremen, Germany).

According to IUPAC standards, isotope ratios are reported as delta (δ) values relative to international reference materials: Vienna−Pee Dee Belemnite (V−PDB) for $\delta^{13}C$, Air for $\delta^{15}N$, and Vienna−Standard Mean Ocean Water (V−SMOW) for $\delta^{2}H$ and $\delta^{18}O$. The delta values are calculated using the following formula (Prohaska et al. 2022):

$$\delta_{ref}\left(iE/^{j}E, sample\right) = \left[\frac{R\left(iE/^{j}E, sample\right)}{R\left(iE/^{j}E, ref\right)}\right] \quad (1)$$

where ref is the international measurement standard, sample is the analysed sample and $^{i}E/^{j}E$ is the isotope ratio (R) between heavier and lighter isotope. The delta values are multiplied by 1000 and expressed commonly in units "per mil" (‰) or, according to the International System of Units (SI), in unit 'milliurey' (mUr).

All measurements were performed in duplicate. For $\delta^{13}C$ and $\delta^{15}N$, values were calibrated against in−house protein standards that were themselves referenced to international standards: NBS−22 fuel oil ($\delta^{13}C$

Table 1
Description of the berberine samples used for stable isotope ratio analysis and experimental parameters (mean, standard deviation (SD), minimum and maximum values, low and high threshold value 95 %) calculated for $\delta^{13}C$ (‰, vs. V-PDB), $\delta^{15}N$, (‰, vs. AIR), $\delta^{2}H$ and $\delta^{18}O$ (‰ vs. V-SMOW).

Origin	Sample	$\delta^{13}C$ (‰, vs V-PDB)	$\delta^{15}N$ (‰, vs AIR)	$\delta^{2}H$ (‰, vs V-SMOW)	$\delta^{18}O$ (‰, vs V-SMOW)
Natural from *Berberis aristata*	BN-1	-32.3	-3.6	-91.2	9.2
	BN-2	-32.0	-4.1	-83.5	7.7
	BN-3	-32.3	-2.9	-86.8	7.9
	BN-4	-32.5	-3.3	-94.7	4.9
	BN-5	-32.6	-3.0	-92.4	4.4
	BN-6	-32.6	-3.9	-94.5	4.4
	BN-7	-32.8	-3.6	-93.6	4.1
	BN-8	-32.9	-2.8	-94.6	1.7
	BN-9	-32.8	-3.2	-100.5	-1.1
	BN-10	-32.7	-3.4	-96.0	-0.8
	BN-11	-32.6	-3.3	-102.3	1.0
	BN-12	-32.9	-2.7	-96.7	0.5
	BN-13	-33.0	-2.5	-101.9	3.7
	BN-14	-32.5	-2.6	-98.7	3.1
	BN-15	-32.5	-2.6	-97.6	2.6
	BN-16	-32.6	-2.6	-97.9	2.5
	BN-17	-32.7	-2.9	-93.0	6.6
	BN-18	-32.7	-2.8	-92.0	6.5
	BN-19	-32.6	-2.8	-92.0	6.4
	BN-20	-32.6	-2.8	-94.0	6.8
	BN-21	-32.9	-3.2	-96.0	6.9
	BN-22	-32.2	-4.2	-75.9	5.5
	Mean	**-32.6**	**-3.1**	**-93.9**	**4.3**
	SD	**0.2**	**0.5**	**6.0**	**2.9**
	minimum value	**-33.0**	**-4.2**	**-102.3**	**-1.1**
	maximum value	**-32.0**	**-2.5**	**-75.9**	**9.2**
	High limit 95 %	**-32.1**	**-2.1**	**-82.0**	**10.0**
	Low limit 95 %	**-33.1**	**-4.1**	**-105.8**	**-1.4**
Synthetic berberine	BS-1	-30.5	-2.4	-98.0	-2.1
	BS-2	-30.5	-2.2	-96.0	-3.1
	BS-3	-30.5	-2.4	-94.9	-1.4
	BS-4	-30.5	-2.3	-91.8	-1.9
	BS-5	-30.3	-2.9	-76.6	-2.1
	BS-6	-30.1	-2.5	-73.6	-2.1
	BS-7	-30.6	-5.8	-66.0	-1.4
	BS-8	-30.1	-3.7	-45.1	-3.3
	BS-9	-30.1	-0.8	-41.3	0.5
	BS-10	-30.0	-1.4	-4.9	4.5
	BS-11	-30.1	-1.7	-4.6	4.3
	BS-12	-29.7	-2.6	-3.1	-1.9
	BS-13	-30.0	-1.5	-3.0	4.0
	Mean	**-30.2**	**-2.5**	**-53.8**	**-0.5**
	SD	**0.3**	**1.2**	**38.9**	**2.8**
	minimum value	**-30.6**	**-5.8**	**-98.0**	**-3.3**
	maximum value	**-29.7**	**-0.8**	**-3.0**	**4.5**

= −30.03 ‰), IAEA–CH–6 ($\delta^{13}C$ = −10.45 ‰), USGS−40 L−glutamic acid ($\delta^{13}C$ = −26.39 ‰, $\delta^{15}N$ = −4.52 ‰), and IAEA−NO3 potassium nitrate ($\delta^{15}N$ = +4.70 ‰).

The $\delta^{2}H$ and $\delta^{18}O$ values were referenced to CBS (Caribou Hoof Standard: $\delta^{2}H$ = −157 ± 2 ‰; $\delta^{18}O$ = +3.8 ± 0.1 ‰) and KHS (Kudu Horn Standard: $\delta^{2}H$ = −35.3 ± 1 ‰; $\delta^{18}O$ = +20.3 ± 0.2 ‰) using a linear calibration equation and a comparative equilibration protocol (Wassenaar and Hobson, 2003). These keratin−based standards were selected due to the lack of an internationally recognized organic standard matrix similar to berberine.

Measurement uncertainty, expressed as standard reproducibility (multiplied by a coverage factor of 2), was found to be < 0.3 ‰ for $\delta^{13}C$ and $\delta^{15}N$, < 1 ‰ for $\delta^{18}O$, and < 3 ‰ for $\delta^{2}H$.

2.3. Statistics

Statistical analysis of the isotopic data was conducted using Statistical software (Statistica®, StatSoft (USA), version 14.0.1.25). The normality of the data distribution was assessed using both the Kolmogorov−Smirnov and Lilliefors tests. To determine statistically significant differences between groups, a one−way analysis of variance (ANOVA) was applied, followed by Tukey's post−hoc test for multiple comparisons. A p−value < 0.05 was considered indicative of statistical significance. Pearson's correlation coefficient was used to evaluate the linear relationship between pairs of variables.

3. Results and discussion

The isotopic data obtained for both natural, synthetic and commercial berberine samples are reported in Table 1.

3.1. Carbon stable isotope ratio

To date, limited data for direct comparison are available since only one study in the scientific literature reported the $\delta^{13}C$ for the plant *Berberis vulgaris* ($\delta^{13}C$ = −27.6 ‰) (Khatri et al., 2021). Nevertheless, similar $\delta^{13}C$ values have been observed in other plants belonging to C_3 species, such as turmeric $\delta^{13}C$ = −30.9 ‰ (Perini et al., 2023), red yeast rice $\delta^{13}C$ = −30.7 ‰ (Perini et al., 2017), Chinese tea $\delta^{13}C$ = −32.4 ‰ (Dunbar and Wilson, 1982), blueberry $\delta^{13}C$ = −30.1 ‰ (Khatri et al., 2021), cinnamon ($\delta^{13}C$ = −31.1 ‰ (Khatri et al., 2021) and java tea $\delta^{13}C$ = −30.4 ‰ (Khatri et al., 2021), further supporting the findings of this study.

Table 1 reports the $\delta^{13}C$ values measured in twenty−two natural berberine samples from *Berberis aristata* (BN−1 to BN−22, Table 1). The values range from −33.0 ‰ to −32.0 ‰, a lower $\delta^{13}C$ range than that observed in products deriving from C_3 photosynthetic species, including *Berberis aristata*, which generally exhibit $\delta^{13}C$ values between −30 ‰ and −23 ‰ (O'Leary, 1988; Perini et al., 2024). This discrepancy may be attributed to isotopic fractionation taking place during berberine

Table 2
Description, isotopic results $\delta^{13}C$ (‰. vs. V-PDB), $\delta^{15}N$ (‰. vs. AIR), $\delta^{2}H$ (‰ vs. V-SMOW), $\delta^{18}O$ (‰ vs. V-SMOW) and final evaluation (based on the proposed $\delta^{13}C$ threshold limit) for berberine samples purchased on the market. The price ratio is calculated as the product price divided by the price of the most expensive product on the market at the time of the study (90 USD/Kg in September 2024).

Origin	Sample	$\delta^{13}C$ (‰. vs V-PDB)	$\delta^{15}N$ (‰. vsAIR)	$\delta^{2}H$ (‰. vs V-SMOW)	$\delta^{18}O$ (‰. vs V-SMOW)	Price ratio	Evaluation
Commercial samples	BC-1	-33.3	-1.7	-95.0	6.0	0.9	Natural
	BC-2	-30.3	-3.6	-44.6	6.6	0.7	Synthetic
	BC-3	-30.7	-2.6	-75.5	9.5	0.6	Synthetic
	BC-4	-32.1	-4.0	-83.3	8.8	0.8	Natural
	BC-5	-29.9	-4.2	-85.1	2.5	0.6	Synthetic
	BC-6	-29.5	-5.3	-70.5	3.4	0.7	Synthetic
	BC-7	-32.9	-1.7	-103.4	6.4	0.7	Natural
	BC-8	-30.9	-2.7	-31.9	3.0	0.7	Synthetic
	BC-9	-29.1	-2.7	-21.0	10.3	0.6	Synthetic

biosynthesis into the plant (O'leary et al., 1992), a phenomenon previously observed in natural caffeine, which shows $\delta^{13}C$ values ranging from −32 ‰ to −25 ‰ (Weckerle et al., 2002). Factors such as CO_2 concentration, water availability and light intensity, differences in leaf lifespan, stomatal conductance and other physiological characteristics as well as the difference in type of the enzymes involved in the synthesis of different molecules can influence their specific isotopic composition.

Fully synthetic molecules are usually produced from petrochemical sources like fuel oil, which shows a broad $\delta^{13}C$ range (–35 ‰ to –19 ‰), averaging around –30 ‰ (Degens, 1969). In comparison, thermogenic natural gas has an average $\delta^{13}C$ value of –40 ‰, while biogenic gas is even more depleted, averaging –65 ‰. Conversely, the synthetic berberine samples considered in the present study and obtained through various and undeclared synthetic routes are characterized by significantly higher (i.e., less negative) $\delta^{13}C$ values, ranging from −30.6 ‰ to −29.7 ‰ (BS−1 to BS−13, Table 1). Some natural pharmacologically active compounds have already been reported to have higher $\delta^{13}C$ values with respect to the synthetic counterpart. For example, synthetic curcuminoid complexes showed an average $\delta^{13}C$ of −27.6 ‰, while their natural counterparts averaged −29.8 ‰ (Perini et al., 2023), highlighting an isotopic shift similar to that found for berberine in the present study. The significant difference between natural and synthetic berberine provides a useful criterion for distinguishing between the two groups. Considering the threshold authenticity limit as $\delta^{13}C_{MEAN}$ 2σ, a natural sample of berberine from *Berberis aristata* can be considered authentic when $\delta^{13}C < -32.1$ ‰ (see Table 2).

3.2. Nitrogen stable isotope ratio

Synthetic berberine samples exhibit $\delta^{15}N$ values ranging from −5.8 ‰ to −0.8 ‰, whereas those of natural origin fall within a narrower range, between −4.2 ‰ and −2.5 ‰. Although there is a slight difference in the average values between the two groups, their ranges partially overlap. As a result, $\delta^{15}N$ does not represent a reliable parameter for distinguishing between synthetic and naturally derived berberine. The overlap reduces its discriminatory power in authenticity testing and source verification for this compound.

Generally, the nitrogen included in synthetic compound structures does not come from fossil materials directly. Instead, it is incorporated into the final molecule through chemical synthesis, often involving the use of plant−based precursors. For instance, in the synthetic route described by Tajiri et al. (Tajiri et al., 2021) the compound piperonal, a naturally occurring aromatic aldehyde extracted from plants such as dill, vanilla, violets, and black pepper, is combined with the precursor isoquinoline. Isoquinoline, in turns, is synthesized from benzaldehyde and aminoacetal, which may derive from either fossil−based or natural sources. Complex or unknown synthetic pathways can make it difficult to determine the actual source of the nitrogen included into the molecular structure. Additionally, the bulk $\delta^{15}N$ may not reflect a purely fossil or biological source but rather a mixture, depending on the production methods of the precursors.

Limitations in the use of $\delta^{15}N$ as a diagnostic marker have already been reported in the isotopic analysis of other compounds. In a study on caffeine, the nitrogen isotopic composition failed to provide clear differentiation between synthetic and natural forms (Dunbar and Wilson, 1982). Instead, the $\delta^{15}N$ proved to be more valuable in other contexts, particularly in distinguishing products from organic versus conventional agricultural systems, where nitrogen sources and fertilization methods are more clearly isotopically differentiated (Laursen et al., 2013).

Overall, while $\delta^{15}N$ values provide some information on the synthetic processes and on the nitrogen sources used in berberine production, they are not effective for definitively distinguishing between natural and synthetic origins in commercial samples.

3.3. Oxygen and hydrogen stable isotope ratio

The $\delta^{2}H$ and $\delta^{18}O$ values of plants and their different chemical components are primarily influenced by environmental factors such as local temperature and humidity. Higher temperatures and lower humidity levels enhance soil and plant evapotranspiration, leading to the isotopic enrichment of the heavier isotopes ^{2}H and ^{18}O in plant tissues (Barbour, 2007; Ziegler, 1989).

According to the literature, the main factors driving the isotopic variability of precipitation include the temperature effect, also linked to the continental effect (i.e., precipitation becomes more isotopically depleted with increasing distance from the coast), and the altitude, since the isotopic composition of precipitation decreases with lower air temperatures (Ingraham, 1998). As a result, coastal regions typically receive "heavier" precipitation compared to inland areas. Although the $\delta^{2}H$ and $\delta^{18}O$ values of rainwater and plant−transpired water are generally similar, the water contained in fresh plant tissues (e.g., grass) is significantly enriched in heavy isotopes compared to the groundwater taken up through the roots (Dawson, 1993; Dawson and Ehleringer, 1998)

Although the exact geographic origin of the berberine samples considered in the present study is not available, it is known that they originate from Chinese and Indian regions. The wide isotopic variability, ranging from −102 ‰ to −76 ‰ for $\delta^{2}H$ and from −1.1 ‰ to + 9.2 ‰ for $\delta^{18}O$, can therefore be explained by the extensive sampling area considered. The two countries differ in average precipitation isotope values, having China generally more negative values ($\delta^{2}H \simeq -50$ ‰ and $\delta^{18}O \simeq -5$ ‰) than India ($\delta^{2}H \simeq -22$ ‰ and $\delta^{18}O \simeq -2$ ‰) (data obtained from the database http://waterisotopes.org). The variability of the precipitation isotopic values finally results in differences in plant−derived products, such as berberine.

Interestingly, the $\delta^{2}H$ and $\delta^{18}O$ of berberine extracted from *Berberis* were not strongly correlated ($R^2 = 0.34$), in disagreement to what is typically observed in other plant matrices. This lack of correlation may be attributed to the specific biosynthetic pathways of berberine synthesis, which may involve different patterns for hydrogen and oxygen isotopic fractionation.

In contrast, synthetic berberine shows a wide range of $\delta^{2}H$ and $\delta^{18}O$ values ($\delta^{2}H$ from −98 ‰ to −3 ‰ and $\delta^{18}O$ from −3.3 ‰ to +4.5 ‰), partially overlapping with the natural product values. While $\delta^{18}O$ does not serve as a reliable distinguishing factor between natural and synthetic origins, $\delta^{2}H$ offers strong discrimination in certain situations. Some synthetic samples (BS−10 to BS−13, Table 1) showed significantly enriched $\delta^{2}H$ values compared to their natural counterparts, a trend also noted in other compounds such as vanillin. The mentioned cases represent an exception, since in all other commercial and synthetic samples (BC and BN, Table 1 and 2) $\delta^{2}H$ values are more negative and indistinguishable from those of natural berberine. This inconsistency is likely due to the use of different synthetic routes in berberine production. Although the catechol−based synthesis pathway is the most common, as reported in the method CN109651361 patented by the Shenyang University of Chemical Technology in 2019, alternative routes starting from other precursors are also documented.

Therefore, $\delta^{2}H$ can serve as a preliminary marker for synthetic origin: values above −76 ‰ are indicative of synthetic berberine, while values below this limit require further investigation using $\delta^{13}C$, which remains the most reliable discriminating parameter.

3.4. A market overview

To assess the market situation, a selection of commercially available berberine products labelled as natural was analysed (Table 2). The sampling strategy prioritized the lowest-priced products, as a lower cost may indicate a higher risk of adulteration. As shown in Fig. 2, most of the samples (6 out of 9) exhibited $\delta^{13}C$ values, and in some cases also $\delta^{2}H$ values, falling within the typical range of synthetic berberine. The mentioned isotopic values were identified in the previous sections as the

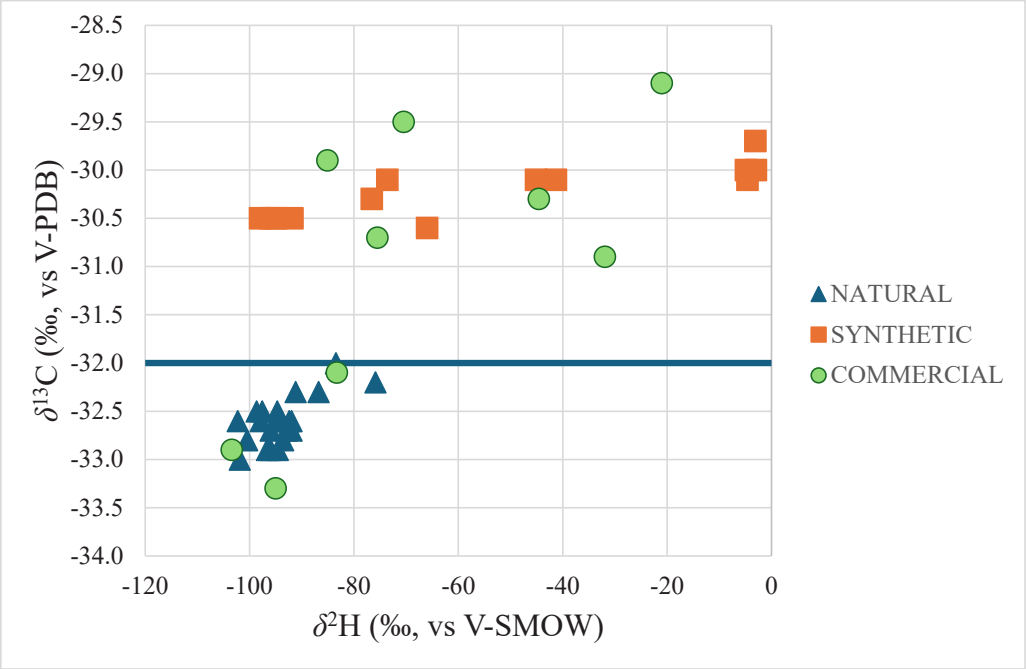

Fig. 2. δ^2H vs δ^{13}C of natural berberine (extracted from *Berberis aristata*), of synthetic berberine and of the commercial samples. The line defines the 95 % threshold limit for authentic natural berberine from *Berberis aristata*.

only reliable discriminant parameters between natural and synthetic berberine. In contrast, δ^{15}N and δ^{18}O showed overlapping distributions between natural and synthetic samples and have therefore limited discriminatory power; for this reason, they will not be further discussed in the context of commercial products. Among the tested parameters, δ^{13}C proved to be the most effective for distinguishing *Berberis*-derived berberine from its synthetic counterpart, clearly highlighting the difference between the two sources.

Information on commercial brands could not be disclosed due to confidentiality constraints. According to supplier declarations, all commercial samples were labelled as originating from Southeast Asia; however, this indication was too generic to support a meaningful geographic interpretation. To provide additional context without compromising confidentiality, relative price information was included, expressed as the ratio between the price of each sample and that of the highest-priced berberine product available on the market at the time of the study (September 2024) (Table 2). Notably some products priced substantially lower than the highest-priced reference coincided with

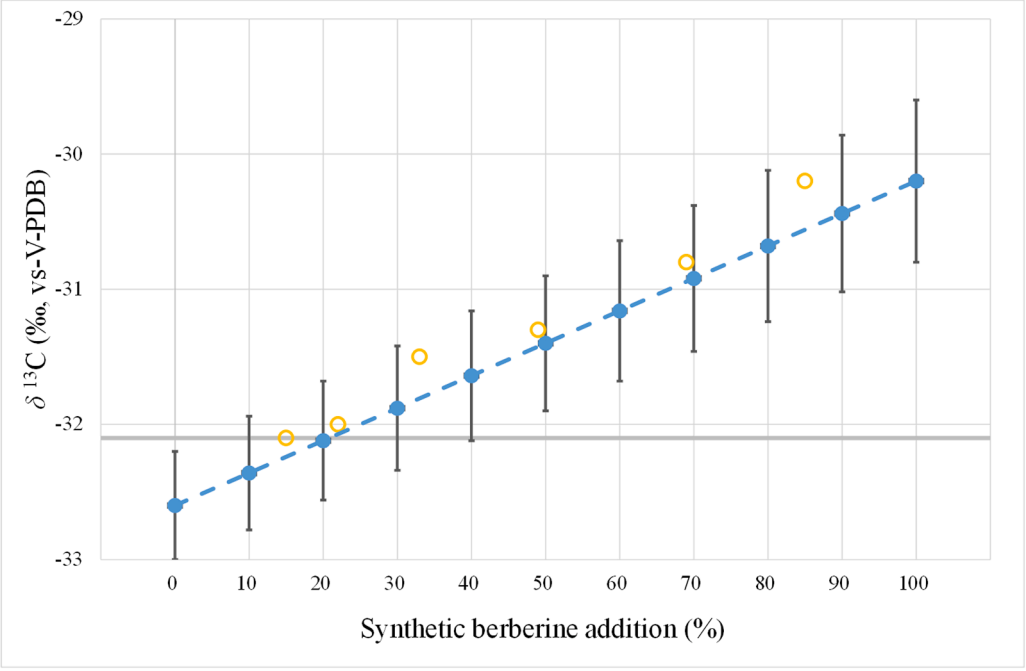

Fig. 3. Variations in the δ^{13}C values of natural berberine from *Berberis aristata* with the addition of synthetic berberine. The grey line defines the 95 % threshold limit for authentic natural berberine. Blue markers: mean value. Bars: 95 % confidence limit. The orange markers represent the values obtained from a sample of natural berberine spiked with a synthetic berberine.

samples identified as adulterated based on their isotopic signatures.

3.5. Addition of synthetic berberin to a natural selected sample

Since $\delta^{13}C$ was identified as the most effective paramenter in the discrimination of natural and synthetic berberin, the average values were used to calculate the proportion of synthetic berberine added as adulternat to natural berberine. A graphical representation of the estimated $\delta^{13}C$ values resulting from the addition of synthetic berberin to a natural one was also provided (Fig. 3, blue markers). The x−axis displays the addition of synthetic berberine, ranging from 0 % (pure natural berberine) to 100 % (pure synthetic berberine). The y−axis shows the estimated mean isotopic values of each mixture, calculated as the weighted average of the mean $\delta^{13}C$ values of the natural and synthetic groups defined in Table 1, according to their respective proportions. Error bars were calculated as standard deviations corrected using the appropriate Student's t−value. The standard deviations were derived based on the principles of error propagation for the sum of independent variables, with each group's standard deviation scaled according to its percentage contribution to the mixture. To test the validity of the calculations, six spiked berberin samples were prepared by adding a growing percentage (from 15 % to 85 %) of synthetic berberine ($\delta^{13}C = -29.7$ ‰) to a natural selected sample (with $\delta^{13}C = -32.6$ ‰). The $\delta^{13}C$ values of the 6 spiked samples are shown as orange markers in Fig. 3.

The isotopic analysis of $\delta^{13}C$ proved to be a robust and sensitive tool for detecting the addition of synthetic berberine to the natural product derived from *Berberis aristata*. Notably, when the proportion of synthetic berberine in the mixture reached a percentage between 20 % and 30 %, the spiked samples showed $\delta^{13}C$ values falling beyond the limit established for natural berberine in Section 3.1 (Fig. 3), providing clear evidence of fraudulent addition.

4. Conclusion

This study highlights the effectiveness of stable isotope ratio analysis as a tool to authenticate the origin of berberine in commercial preparations. Among the isotopic parameters evaluated, $\delta^{13}C$ emerged as the most discriminant parameter between natural and synthetic sources, as it allows the differentiation between plant−derived and chemically synthesized berberine. The $\delta^{2}H$ values showed potential as a complementary marker, especially when synthesis routes introduce significant isotopic enrichment. On the other hand, $\delta^{15}N$ and $\delta^{18}O$ were less reliable due to the overlap of synthetic and natural berberine values and to a greater isotopic variability.

A survey on commercial berberine supplements revealed that most low−cost products labelled as "natural" likely contain synthetic berberine, confirming the presence of economically motivated adulteration on the market. These findings call for stricter quality control protocols and the routine implementation of isotope−based authentication methods in regulatory and industrial settings to protect consumers and preserve the integrity of clinical research relying on natural formulations.

CRediT authorship contribution statement

Matteo Perini: Writing – review & editing, Writing – original draft, Resources, Methodology, Data curation, Conceptualization. **Elena Apelganets:** Formal analysis. **Silvia Pianezze:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Clizia Lacerenza:** Writing – original draft, Investigation, Conceptualization. **Andrea Poletti:** Writing – original draft, Data curation, Conceptualization. **Roberto Larcher:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to express our sincere gratitude to Dr. Giorgio Cerasa and Vivatis Pharma Italia S.r.l. for their invaluable support, including analytical assistance and the provision of samples, which greatly contributed to the success of this work.

Data availability

Data will be made available on request.

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