Published online: 2020-10-21

Profiling and Quantification of the Key Phytochemicals from the Drumstick Tree (*Moringa oleifera*) and Dietary Supplements by UHPLC-PDA-MS

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Key words
Moringa oleifera, Moringaceae, niazirin, flavonoids, lignans, UPLC-PDA-MS

received February 24, 2020
accepted after revision August 13, 2020
published online October 21, 2020

Bibliography
Planta Med 2021; 87: 417–427
DOI 10.1055/a-1240-6186
ISSN 0032-0943
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Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

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Supplementary material is available under https://doi.org/10.1055/a-1240-6186

ABSTRACT

Moringa oleifera is known as a drumstick tree and is cultivated in the subtropics and tropics. It exhibits antihypertensive and antidiabetic effects. An ultra-high-performance liquid chromatography method was developed for the determination of 9 phytochemicals in *M. oleifera* leaves and marketed products. The efficient separation was achieved within 7 min with a temperature of 45 °C by using a C-18 column as the stationary phase and water/acetonitrile with 0.05% formic acid as the mobile phase. The method was validated for linearity, repeatability, limits of detection, and limits of quantification. The limits of detections of phenolic compounds **1**–**9** were as low as 0.2 µg/mL. The photodiode array detector at 220 and 255 nm wavelengths was recruited for quantification. The key phytochemicals were detected in the range of 0.42 to 2.57 mg/100 mg sample weight in 13 dietary supplements. This study considers the quantitative analysis for lignans in *M. oleifera* for the first time. Isoquercitrin (**5**) and quercetin 3-*O*-(6-*O*-malonyl)-*β*-D-glucopyranoside (**6**) predominates the leaves of *M. oleifera* with inherent degradable nature detected for compound **6**. Niazirin (**2**) was detected in amounts between 0.010–0.049 mg/100 mg while compound **1** was undetectable and potentially an artifact because of the fractionation process. The characterization and confirmation of components were achieved by liquid chromatography-electrospray ionization-mass spectrometry with extractive ion monitoring for the positive and negative ion modes. The developed and validated method is robust and rapid in the conclusive quantification of phytochemicals and authentication of the *Moringa* samples for quality assurance.

Introduction

Moringa oleifera Lam. (synonym: *Moringa pterygosperma* Gaertn.) is known as drumstick or horseradish tree and belongs to the monogeneric genus *Moringa* beneath Moringaceae family. Due to multiple medicinal benefits, *M. oleifera* has so far become the most commonly used among 13 species [1, 2]. India and Pakistan are described as the origin of this species, but it is nowadays cultivated beyond its native range in subtropical and tropical provinces such as Flora of West Tropical Africa. *M. oleifera* attracted the at-

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tention of the scientific community in the last 2 decades because of the traditional medicine claims. For instance, Ayurvedic medicine mentions that *M. oleifera* can prevent 300 diseases [3,4]. The seeds oil is applied externally for rheumatism and gout while the roots and fruits are prescribed for ascites, splenomegaly, and hepatomegaly. The flowers are administered for its aphrodisiac action while leaves are considered as food [5]. It has been called the miracle vegetable because all parts of *Moringa,* such as pods, leaves, roots, and seeds, have been exploited to treat a variety of ailments [6]. Besides antioxidant properties, it shows antihypertensive, antimicrobial, antidiabetic, anticancer, anti-inflammatory, and anti-ulcerogenic activities alongside immunomodulatory, cardioprotectant, and hepatoprotectant effects [7–10].

Roughly, 110 compounds were reported from the *Moringa* genus including flavonoids, glucosinolates, isothiocyanates, alkaloids, terpenoids, and lignans, and a multitude of therapeutic benefits for the *Moringa* species is likely associated with this chemical diversity [11,12]. The abundant chemical classes reported are glucosinolates (thioglucoside conjugates) and flavonols, predominantly quercetin, kaempferol, and isorhamnetin glycosides [13].

Exploiting an array of well-characterized secondary metabolites as quality standards, isolated from an authentic sample of *M. oleifera*, aids in developing a chemical fingerprint profile, which serves as a guideline for accurate quantitative analysis of these phytochemicals in botanical supplements claimed to contain *M. oleifera.* Prior studies focused mostly on the qualitative analysis of *M. oleifera* compositions using structural interrogation by mass spectrometry. For instance, an extraction method to obtain a phenolic compounds-rich extract from *M. oleifera* leaves was optimized, and mainly 30 flavonoids and phenolic acids among 59 components were characterized for the first time [14]. Also, an untargeted metabolomic study based on multivariate statistical analysis was performed between Chinese and Indian ecotypes of *M. oleifera* leaves and identified 118 shared among 122 characterized components [15]. Owing to the biogenetical capabilities, a comparative study reported the glycosylation complexity of *M. oleifera* compared with *Moringa ovalifolia* Dinter & A. Berger, which were glycosylated with only rutinoside [16]. Other researchers identified 39 components from the acetone extract of *M. oleifera* leaves, with higher contents determined for acetylglucomoringin, feruloylquinic acid, and caffeoylquinic acid using UPLC-Q-orbitrap-MS^2 [17]. However, the following quantitative analysis was mainly based on a semi-quantitative approach, which might provide inaccurate results. Limited quantification analysis was also achieved, such as the determination of nitrile glycosides, niaziridin, and niazirin contents in leaves and pods [18], and the quantification of crypto-chlorogenic acid, isoquercitrin, and astragalin in *M. oleifera* leaves [19]. Comprehensive analysis of tissues including leaves, barks, and roots of *M. oleifera* and *Moringa stenopetala* (Baker f.) Cufod. for profiling glucosinolates and phenolics was also performed [20]. However, the enormous suggestibility of UPLC compared with HPLC will enable improving the resolution and reducing the total analysis time (40 mins.). Herein, it is crucial to develop an accurate, rapid, and economic analytical method to overcome the previous hitches, including potentially misleading results provided by semi-quantitative analysis alongside delay analysis time. In our continued pursuit to explore the quality and safety of botanical ingredients, this study aims to scrutinize the phenolic profile of *M. oleifera* via developing and validating a robust, suitable, and straightforward analytical fingerprinting approach that potentially aids in the identification and quantification of phytochemicals and authentication of the *Moringa* samples for quality assurance.

The current study was intended for the qualitative and quantitative analysis of 9 selected phenolic glycosides: 2 glucosinolate-derived hydrolysis products, 4 flavonoids, and 3 lignans in *M. oleifera* using ultra-high-performance liquid chromatography-photodiode array-mass spectrometry (UHPLC-PDA-MS). The method is also applied to dietary supplements claiming to contain *M. oleifera*. The 9 standard compounds used as chemical markers were methyl-4-(*α*-L-rhamnopyranosyloxy) benzyl carbamate (**1**), niazirin (**2**), (+)-isolariciresinol-3a-*O*-*β*-D-glucopyranoside (**3**), rutin (**4**), isoquercitrin (**5**), quercetin-3-*O*-(6-*O*-malonyl)-*β*-D-glucopyranoside (**6**), laricirесinol-9-*O*-*β*-D-glucopyranoside (**7**), (+)-pinoresinol-4-*O*-*β*-D-glucopyranoside (**8**), and astragalin (**9**) as illustrated in ▶ **Fig. 1**.

Results

The plant samples were composed of complex mixtures of components, and 3 extraction techniques were employed. The authenticated plant sample (#18332) was extracted separately with methanol, 80% methanol, and 70% methanol in water exhaustively to quest the optimal extraction solvent. The abundance values for compounds **1**–**9** in the sample extracted with 70% methanol were found to be higher than the samples extracted with pure methanol or 80% methanol in water.

Optimized chromatographic conditions were achieved after several trials with acetonitrile, methanol, and water in different proportions for the mobile phase. A mobile phase containing water and acetonitrile, both containing formic acid, with a constant flow rate at 0.40 mL/min on an HSS T3, 1.8 µm, 2.1 × 100 mm using gradient elution at a fixed column temperature of 45 °C, was deemed the optimal separation condition for the determination of compounds **1**–**9** in various samples. The different columns tried were Acquity UPLC BEH Shield RP18, 1.8 um, 2.1 × 100 mm and Cortecs UPLC C18, 1.6 um, 2.1 × 100 mm. Each provided a different combination of silanol activity, hydrophobicity, hydrolytic stability, and chemical interaction with the analytes. Among these, the HSS T3, 1.8 µm, 2.1 × 100 mm resolved peaks **1**–**9** adequately. Other columns could not resolve the lignin derivatives **7** and **8** satisfactorily under the same conditions.

Quantitative determination of 9 compounds in various samples was achieved using the UHPLC-PDA method, and results were expressed as mg/100 mg on a dry sample weight basis. The developed method was validated regarding sensitivity (limit of detection [LOD] and limit of quantification [LOQ]), linearity, intra-day and inter-day precision for 3 consecutive days, accuracy, specificity, stability, system suitability, and robustness. These validation parameters enabled us to investigate the suitability of the method for routine analysis.

The 7-point calibration curve exhibited a linear correlation between concentration and peak area. Calibration data indicated the linearity ($r^2 > 0.998$) of the detector response for compounds **1**–**9**

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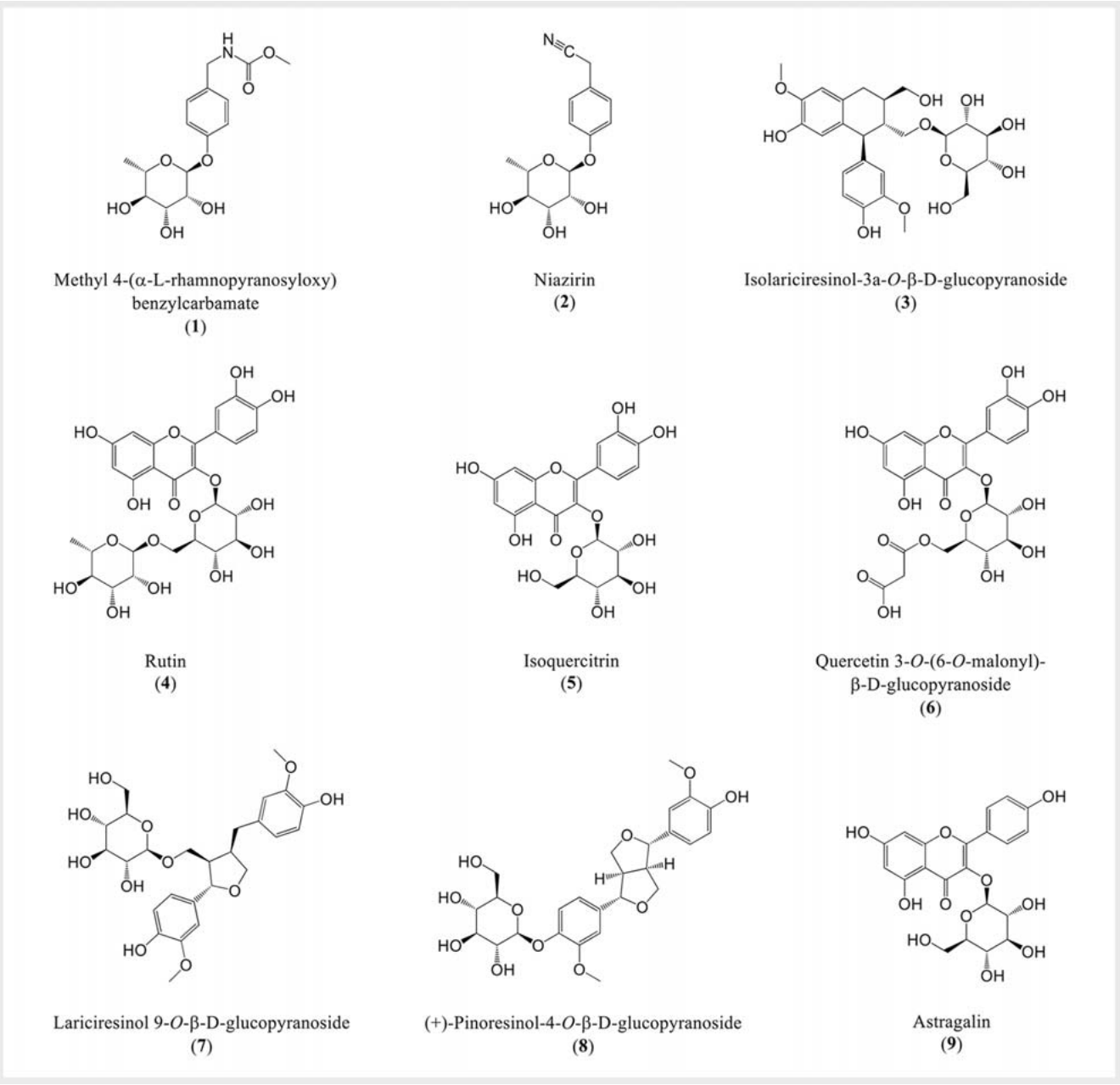

▶ **Fig. 1** Chemical structures of reference marker phytochemicals **1**–**9** from *Moringa oleifera*.

from 1 to 100 µg/mL, with additional concentration points (200–500 µg/mL) considered for compounds **5**–**6** and **8**. The limits of detection were below 2 µg/mL for compounds **1**–**9**. All samples and standards were injected in triplicate.

Intra- and inter-day variation for this study was determined for the authenticated *M. oleifera* plant sample (#18332), and relative standard derivation (RSD) was lower than 6% except for compound **6**. The analysis was conducted 3 times on 3 different days, and each run was repeated in triplicate. The intra-day RSD for the replicates for compounds **1**–**9** were between 0.68 and 5.00% while RSD for the day-to-day replicates were between 2.9 and 5.7%. The intra-day RSD for the replicates for compound **6** was 2.83, 4.28, and 0.85 for day 1, 2, and 3, respectively. However, the inter-day RSD for the replicates was 9.2.

The method accuracy for the related compounds was assigned by spiking a known amount of compounds **1**–**9**. Sample #18332 was extracted exhaustively 5 times, dried, and spiked with a known amount of standard compounds, then extracted and analyzed using the same method of sample preparation. The accuracy of developed method was evaluated in duplicate. The recovery of analytes ranged from 93.58 to 105.47%.

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Discussion

According to the World Health Organization (WHO), the described risks associated with traditional medicines comprise undesirable side effects, exposure of the the individual to unreliable information, and the consumption of inferior quality products. Bearing in mind the claims of medical effectiveness, tremendous economic impact, and the possibility of herb-drug interaction, there is a necessity to develop and validate an analytical fingerprinting approach for identification and quantification of the phenolic compounds as reference markers in *Moringa* samples. Although several analytical methods have been applied for the qualitative and quantitative analysis of *Moringa*, the poor resolution, delay analysis time, and reduced sensitivity and robustness hampered their suitability for routine applications. This highlights the necessity for robust research aimed at standardizing *Moringa* herbal products and quality control.

M. oleifera is a prolific source for unique secondary metabolites that are biosynthesized by the plant to survive in semiarid regions and adapt to environmental stressors. The predominant chemical classes reported are glucosinolates (thioglucoside conjugates) and flavonols including quercetin, kaempferol, and isorhamnetin glycosides. Previous studies described the complexity of the flavonoid profile for *M. oleifera*, which contains glucosides, rutinosides, malonylglucosides, and acetylglucosides. In addition, fatty acid profiling indicated that the leaves mainly contain palmitic and linoleic acid [13].

The UHPLC-UV separation of 70% methanol extracts of *M. oleifera* at 220 nm are shown in ▶ **Fig. 2**. Optimization strategies were pursued by modifying the temperature, flow rate, mobile phase gradient, and stationary phases. Although increased flow rate positively reduced the analysis time, it simultaneously affected the resolution. All 9 standard compounds were separated within less than 7 min. The peaks of compounds in all samples were identified by comparing the retention time, and UV and MS spectra to the standards, which showed $[M + H]^+$ and $[M + Na]^+$ ions in the positive mode, and $[M - H]^-$ and $[M - H + HCO_2H]^-$ ions in the negative mode (▶ **Table 1**). The analysis was performed on an authenticated plant material (#18332) and 13 dietary supplements. Of the 13 dietary supplement brands tested, all products contained phenolic compounds with varied contents (▶ **Table 2**). The total content of phenolic compounds analyzed (**1**–**9**) was found to be in the range from 0.42 to 2.57 mg/100 mg sample weight for the herbal supplements (▶ **Fig. 3**).

The characterization and confirmation of 9 compounds from plant samples and dietary supplements were achieved by liquid chromatography-mass spectrometry with electrospray ionization (LC-ESI-MS) hyphenated technique coupled with extractive ion monitoring. All compounds of interest displayed the superior response in negative mode ionization. The chromatogram shows no overlapping peaks at the retention time of interest. Also, the mass spectrum exhibited fragmentation behavior that was diagnostic in the characterization of the compounds (▶ **Table 1**). Particularly, the fragmentation pattern was distinctive in the characterization of compound **6,** which was tentatively identified as quercetin 3-*O*-(6-*O*-malonyl)-*β*-D-glucopyranoside based on the molecular ion peak and fragment ions. This compound was confirmed later by a reference standard that was purchased and recruited for the quantitative analysis. The method involved the use of both positive and negative ion modes for compounds **1**–**9.** The standard compounds in the positive ion mode were observed at *m/z* 328.13 $[M + H]^+$ (**1**), 302.18 $[M + Na]^+$ (**2**), 523.15 $[M + H]^+$ (**3**), 611.09 $[M + H]^+$ (**4**), 465.08 $[M + H]^+$ (**5**), 550.88 $[M + H]^+$ (**6**), 523.12 $[M + H]^+$ (**7**), 543.12 $[M + Na]^+$ (**8**), and 449.08 $[M + H]^+$ (**9**), respectively. On the contrary, the standard compounds in the negative ion mode were detected at *m/z* 372.09 $[M - H + HCO_2H]^-$ (**1**), 324.06 $[M - H + HCO_2H]^-$ (**2**), 521.30 $[M - H]^-$ (**3**), 609.18 $[M - H]^-$ (**4**), 463.16 $[M - H]^-$ (**5**), 549.12 $[M - H]^-$ (**6**), 521.27 $[M - H]^-$ (**7**), 519.25 $[M - H]^-$ (**8**), and 447.18 $[M - H]^-$ (**9**), respectively.

Prior studies reported the isolation of compound **1** from the methanol extract of *M. oleifera* [21, 22]. In agreement with other research groups, we isolated this compound from the methanol extract of *M. oleifera* (#18332) in the previous isolation work [23]. However, compound **1** in the plant sample (#18332) and 13 herbal supplements in this analytical work were undetectable. All chemical profiles lacked the peak corresponding to compound **1** based on extractive ion monitoring. Consequently, the absence of compound **1** raised an issue regarding its source. It is uncertain whether this compound is a natural product or an artifact resulting from the extraction and fractionation conditions. Knowing that the glucomoringin is the predominant glucosinolate reported in *M. oleifera*, a plausible myrosinase-dependent formation of compounds **1** and **2** is illustrated in ▶ **Fig. 4**. Generally, cutting *M. oleifera* converts the glucomoringin precursor to free glucose and unstable intermediate followed by rearrangement to biologically active nitrile, niazirin (**2**), and isothiocyanate derivative. This process is catalyzed by the myrosinase enzyme released from an isolated compartment in the plant itself [24]. Because of stability issues, isothiocyanate derivative is vulnerable to further conversion in the presence of nucleophilic, such as a thiol or hydroxy, groups, resulting in thiocarbamate or carbamate derivatives, respectively. Alongside the plausible myrosinase-dependent formation, the absence of compound **1** in multiple chemical profiles of the plant materials extracted with different solvents indicated that compound **1** was either present in traces below the limit of detection (1 µg/mL) or potentially an artifact because of the fractionation conditions. A previous analytical study reported the inability to detect the thiocarbamates and carbamates in *M. oleifera* in agreement with this result [20].

The content of niazirin (**2**) in the leaves of the authentic plant material (0.034 mg/100 mg) was in conformance to what has been reported in the literature (0.038 mg/100 mg) despite the variation of the extraction procedure [18]. However, the herbal supplements analyzed exhibited different chemical profiles, and this reference marker was detected in varying amounts between 0.010–0.049 mg/100 mg. Two samples (#20741 and 20747) contained higher content than other products as 0.097 and 0.468, respectively. This variation could be attributed to several factors such as genetic background, environmental effects, cultivation methods, the tissue used, or the myrosinase abundancy and the degree of activation.

Rutin (**4**) is known for its pharmacological activities and could be a contributor to the health-promoting benefits of several me-

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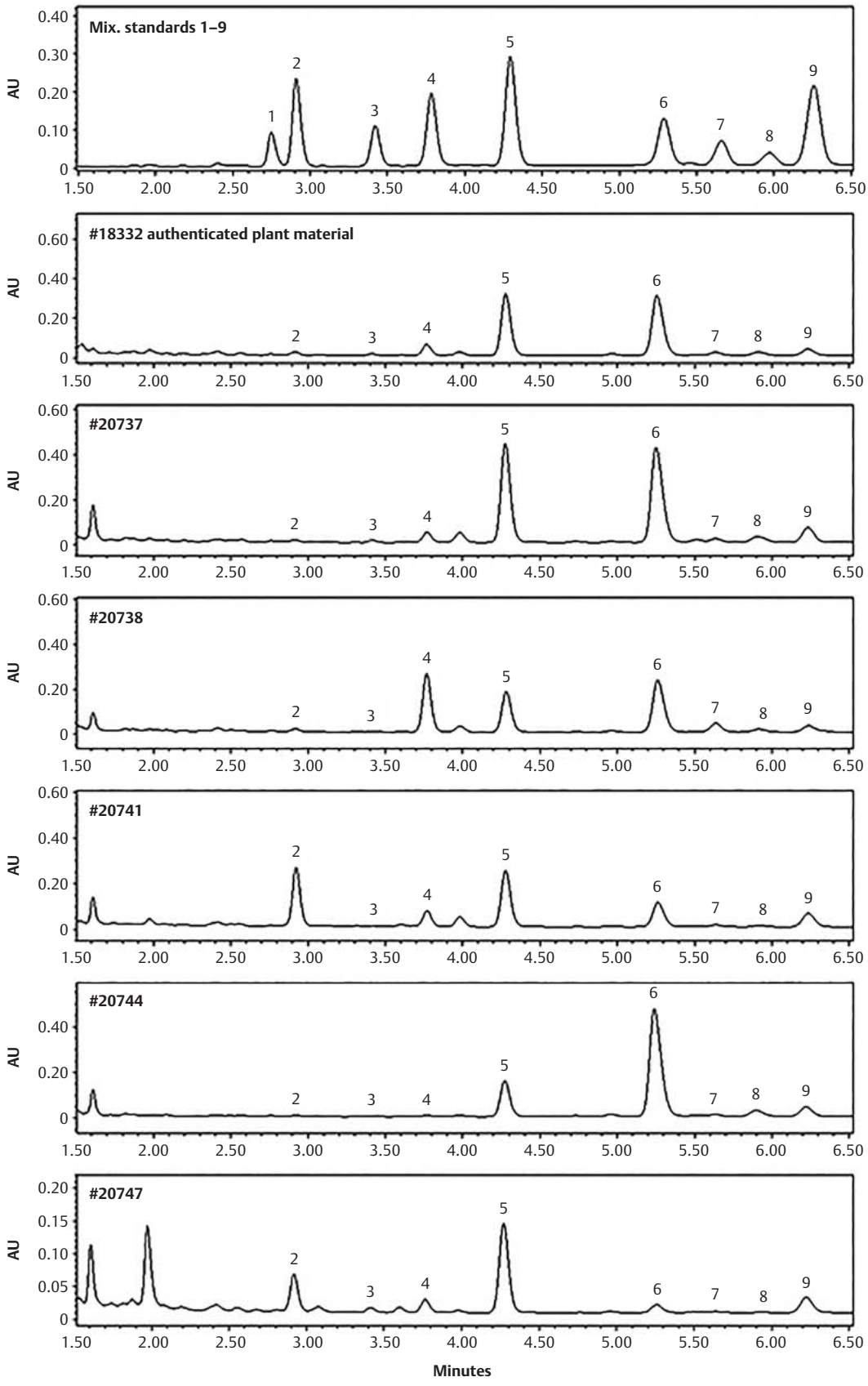

▶ **Fig. 2** UHPLC-UV Chromatograms of a standards mixture (**1–9**), authenticated plant sample (#18332), and selected dietary supplements (#20737, #20738, #20741, #20744, and #20747) at 220 nm.

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▶ **Table 1** Compound name, retention time (min), LOD, LOQ, λ max used for analysis, M.wt, molecular formula, UV and MS spectra of compounds used for analysis of *M. oleifera*.

Sample No.*	Compound Name	t_R (min)	LOD (µg/mL)	LOQ (µg/mL)	λ max used for analysis	M.wt	Molecular formula	UV (nm)	*m/z* $[M + H]^+$/ $[M + Na]^+$	*m/z* $[M - H]^-$/$[M - H + HCO_2H]^-$
1	Methyl 4-(α-L-rhamnopyranosyloxy) benzylcarbamate	2.774	1	5	220	327.33	$C_{15}H_{21}NO_7$	220, 271	328.13	372.09 $[M - H + HCO_2H]^-$
2	Niazirin	2.926	0.5	2	220	279.29	$C_{14}H_{17}NO_5$	220, 271	302.18 $[M + Na]^+$	324.06 $[M - H + HCO_2H]^-$
3	(+)-Isolariciresinol-3a-*O*-β-D-glucopyranoside	3.427	1	5	220	522.54	$C_{26}H_{34}O_{11}$	202, 227, 283	523.15 (frag., 219.10)	521.30
4	Rutin	3.792	0.5	2	255	610.52	$C_{27}H_{30}O_{16}$	203, 255, 354	611.09 (frag., 465.09, 303.08)	609.18
5	Isoquercitrin	4.307	0.2	1	255	464.37	$C_{21}H_{20}O_{12}$	203, 255, 353	465.08 (frag., 303.07)	463.16
6	Quercetin 3-*O*-(6-*O*-malonyl)-β-D-glucopyranoside	5.295	0.5	2	255	550.42	$C_{24}H_{22}O_{15}$	204, 255, 354	550.88 (frag., 303.12)	549.12 (frag., 505.13)
7	Lariciresinol 9-*O*-β-D-glucopyranoside	5.666	1	5	220	522.54	$C_{26}H_{34}O_{11}$	227, 280, 340	523.12 (frag., 219.11)	521.27
8	(+)-Pinoresinol-4-*O*-β-D-glucopyranoside	5.955	2	10	220	520.53	$C_{26}H_{32}O_{11}$	201, 227, 278	543.12 $[M + Na]^+$ (frag., 341.13, 235.09)	519.25 (frag., 357.04)
9	Astragalin	6.277	0.5	2	255	448.38	$C_{21}H_{20}O_{11}$	265, 347	449.08 (frag., 287.08)	447.18

* The number for the reference compounds are corresponding to the elution order by the column.

dicinal plants [25]. Because of the incapability of other plant species to store rutin in large amounts, prior study claims that Ethiopian *Moringa* (*M. stenopetala*) has a superior advantage to be one of the large-scale alternative sources for this widely distributed flavonoid, as its content was reported as 2.3 mg/100 mg [26]. However, rutin (**4**) was determined in the authentic sample of *M. oleifera* at 0.052 mg/100 mg in this study. Except for 3 samples (#20738, 20739, and 20744), which were determined as 0.480, 0.412, and 0.007 mg/100 mg respectively, rutin (**4**) was detected between 0.018 to 0.076 mg/100 mg in other *Moringa* products. Among 12 ecotypes of *M. oleifera* analyzed in a previous study, it was reported that distributions of rutinoside-bearing flavonoids such as quercetin, kaempferol, and isorhamnetin rutinosides were cultivar-dependent [27]. In the current study, the discrepancy and extreme values of the rutin content in the above mentioned 3 samples might be attributed to abiotic and biotic elicitors that influence the cultivation and play significant roles in secondary metabolites production [28].

Although they have been structurally interrogated in preceding studies, this work considers the quantitative analysis for lignan derivatives biosynthesized by *M. oleifera* for the first time [14, 15]. Individually, (+)-isolariciresinol-3a-*O*-β-D-glucopyranoside (**3**), lariciresinol-9-*O*-β-D-glucopyranoside (**7**), and (+)-pinoresinol-4-*O*-β-D-glucopyranoside (**8**) were analyzed qualitatively and quantitatively. Among them, compound **8** was detected as the abundant lignan, and the content in the herbal supplements was varied compared with its counterparts **3** and **7,** whose percentages were around the authentic sample range. According to the quantitative results, it was apparent that dietary supplements (#20738 and 20739) have higher content in rutin (**4**) associated with an increase in lariciresinol-9-*O*-β-D-glucopyranoside (**7**). On the contrary, compounds **7** and **8** were qualitatively detected but under the limit of quantification for the herbal supplement #20747. This product was labeled as *M. oleifera* extract (10 : 1) specifying that the capsule contains the extract instead of the intact leaves. The low abundance values might be associated primarily with the poor extraction method by the manufacturer, assuming that the applied approach was inappropriate to extract the mentioned phytochemicals in measurable yields. A previous study highlighted the impact of the extraction method on lignans and postulated that microwave-assisted extraction was superior in extracting these components exhaustively compared to pressurized liquid extraction [29]. This observation shed light on the significance of good manufacturing practices for quality control in future studies.

Among the analyzed constituents, isoquercitrin (**5**) and quercetin 3-*O*-(6-*O*-malonyl)-β-D-glucopyranoside (**6**) predominate the leaves of *M. oleifera*, which was in agreement with a previous study that reported that glucosinolates alongside the flavonoids,

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▶ **Table 2** Percentage content (%, mg/100 mg sample weight) of 9 phytochemicals in authenticated plant material (#18332) and 13 herbal supplements claimed to contain *M. oleifera*: methyl-4-(*α*-L-rhamnopyranosyloxy) benzyl carbamate (**1**), niazirin (**2**), (+)-isolariciresinol-3a-*O*-*β*-D-glucopyranoside (**3**), rutin (**4**), isoquercitrin (**5**), quercetin-3-*O*-(6-*O*-malonyl)-*β*-D-glucopyranoside (**6**), lariciresinol-9-*O*-*β*-D-glucopyranoside (**7**), (+)-pinoresinol-4-*O*-*β*-D-glucopyranoside (**8**), and astragalin (**9**).

	Compounds. (mg/100 mg sample)									
Code #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
18332	ND	0.034 (2.04)	0.034 (1.36)	0.093 (2.60)	0.499 (2.85)	0.730 (2.83)	0.081 (4.32)	0.252 (2.93)	0.067 (2.47)	1.791
20737	ND	0.023 (1.62)	0.041 (3.24)	0.052 (2.03)	0.702 (3.94)	1.122 (3.21)	0.060 (3.44)	0.445 (3.29)	0.126 (3.48)	2.570
20738	ND	0.026 (1.30)	0.014 (4.68)	0.480 (1.91)	0.277 (1.91)	0.582 (2.00)	0.226 (4.33)	0.182 (1.62)	0.061 (1.81)	1.849
20739	ND	0.022 (5.02)	0.014 (3.94)	0.412 (6.51)	0.460 (6.68)	0.848 (6.33)	0.150 (1.21)	0.262 (3.44)	0.067 (6.63)	2.234
20740	ND	0.031 (0.40)	0.022 (5.78)	0.018 (2.93)	0.438 (2.80)	1.062 (2.81)	0.028 (6.79)	0.348 (1.08)	0.086 (2.89)	2.033
20741	ND	0.468 (3.26)	0.013 (2.73)	0.075 (3.39)	0.392 (3.30)	0.271 (3.33)	0.041 (1.80)	0.101 (2.46)	0.086 (3.29)	1.446
20742	ND	0.027 (1.03)	0.018 (1.20)	0.018 (1.63)	0.521 (1.60)	1.073 (1.63)	0.031 (3.15)	0.431 (2.79)	0.104 (1.48)	2.222
20743	ND	0.021 (3.04)	DUL	0.032 (2.45)	0.361 (2.49)	0.843 (2.47)	0.037 (7.79)	0.284 (1.61)	0.071 (2.51)	1.650
20744	ND	0.010 (1.32)	DUL	0.007 (1.88)	0.247 (3.00)	1.245 (2.91)	0.038 (3.19)	0.414 (1.56)	0.076 (3.31)	2.038
20745	ND	0.049 (1.18)	0.025 (8.44)	0.068 (2.16)	0.378 (2.06)	0.565 (2.12)	0.057 (0.08)	0.202 (4.68)	0.100 (2.05)	1.445
20746	ND	0.028 (2.29)	0.021 (0.70)	0.050 (0.85)	0.330 (0.68)	0.558 (0.67)	0.035 (1.27)	0.199 (0.09)	0.064 (0.65)	1.286
20747	ND	0.097 (3.61)	0.027 (3.15)	0.024 (3.72)	0.203 (4.12)	0.025 (5.02)	DUL	DUL	0.042 (4.25)	0.417
20748	ND	0.034 (1.13)	0.025 (5.63)	0.076 (0.13)	0.611 (1.60)	0.754 (0.85)	0.045 (1.42)	0.220 (1.59)	0.117 (1.38)	1.881
20749	ND	0.038 (5.06)	0.014 (0.46)	0.026 (4.11)	0.407 (4.17)	0.383 (4.18)	0.030 (4.83)	0.169 (5.26)	0.078 (4.00)	1.145

ND: not detected; DUL: detected under LOQs; relative standard deviations are given in parentheses

certainly the aforementioned components, are the abundant phytochemicals detected in the leaves [20]. Due to their distinctive structural elements, flavonoids play an important role as radical scavengers in attenuating the oxidative stress-related diseases [30, 31]. Consequently, the therapeutic potential of *M. oleifera,* such as antioxidant, anti-inflammatory, anticancer, antidiabetic, and cardioprotective effects, might be attributed to the presence of these 2 abundant phytochemicals and other flavonoids [32]. However, 2 products (#20741 and #20747) displayed contradicting patterns for compound **6** with low abundance values detected as 0.271 and 0.025 mg/100 mg, respectively. Product #20741 has a content of nizairin (**2**)–10 times higher than the authentic sample and other herbal supplements. This observation speculates that the product comprises other tissue parts instead of entire leaves, which contributed to the contrasting result. As mentioned earlier, herbal supplement #20747 contained *M. oleifera* extract rather than the intact leaves, and the extraction method might remarkably affect the chemical profile and the percentage of the analyzed phytochemicals.

Knowing that the intra-day RSD for the replicates of compound **6** was 2.83, 4.28, and 0.85 for day 1, 2, and 3, respectively while the inter-day RSD for the replicates was 9.2 enabled us to perceive the inherent degradable nature of compound **6**. This could be a substantial characteristic that affected the quantity of the compound in the authentic sample. Although there was no apparent increase in isoquercitrin (**5**), it is likely that compound **6** converts rapidly to another component, acetylated isoquercitrin, which might undergo further hydrolysis slowly to provide isoquercitrin (**5**). Long-term stability studies in this regard are warranted to illuminate the hydrolyzable character of compound **6** under altered conditions including temperature, pH, and time to highlight the impact of shelf life on quality of products. A previous study stated that the malonyl glycosides of flavonoids in methanol extract were thermolabile, and they were converted to its corresponding flavonoid glycosides when monitored at 60 °C for 24 h by HPLC-MS [33].

In conclusion, the newly developed method is suitable for the quality control and chemical fingerprint analysis for identification

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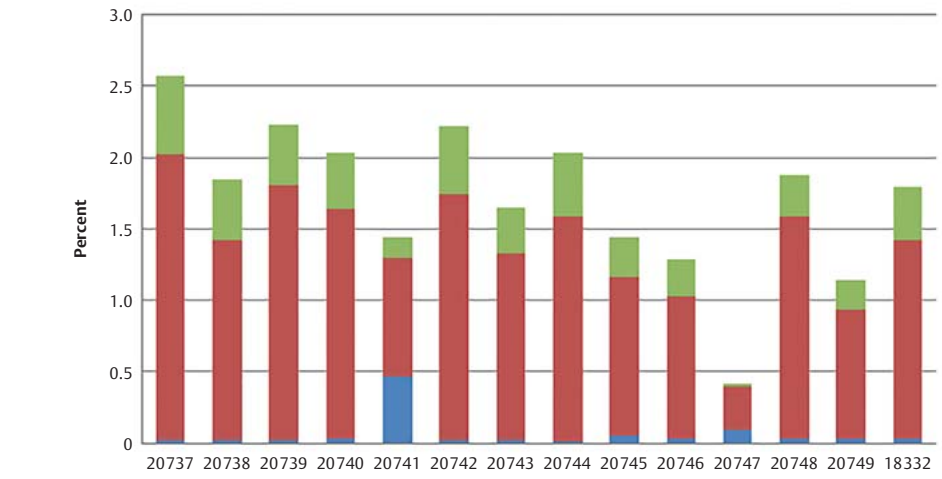

▶ **Fig. 3** Lignans, flavonoids and glucosinolate-derived hydrolysis products (GDHP) analyzed in an authenticated plant sample (#18332) and dietary supplements (#20737, #20738, #20739, #20740, #20741, #20742, #20743, #20744, #20745, #20746, #20747, #20748, and #20749). Lignans and glucosinolate-derived hydrolysis products (GDHP) were quantified at 220 nm while flavonoids were quantified at 255 nm. The analyzed phytochemicals (**1**–**9**) were summed according to chemical classes. Glucosinolate-derived hydrolysis products (GDHP) represent methyl 4-(*α*-L-rhamnopyranosyloxy) benzylcarbamate (**1**) and niazirin (**2**). Lignans represent (+)-isolariciresinol-3a-*O*-*β*-D-glucopyranoside (**3**), lariciresinol 9-*O*-*β*-D-glucopyranoside (**7**) and (+)-pinoresinol-4-*O*-*β*-D-glucopyranoside (**8**). Flavonoids represent rutin (**4**), isoquercitrin (**5**), quercetin 3-*O*-(6-*O*-malonyl)-*β*-D-glucopyranoside (**6**) and astragalin (**9**).

and quantification of compounds in plant samples and dietary supplements claimed to contain *M. oleifera*. UHPLC-PDA-MS method for the determination of phenolic compounds was found to be efficient in providing low retention times and excellent resolution simultaneously. It was validated for all the parameters tested, and 13 dietary supplements were analyzed accordingly. All tested dietary supplements contained phenolic compounds, and the quantities in these brands ranged from 0.42 to 2.57 mg/100 mg sample weight. The difference in the phenolic contents between the authentic plant material and the nutraceuticals stresses the significance of chemical fingerprint profiling and quantitative analysis for quality assurance.

Materials and Methods

Chemicals and plant samples

Secondary metabolites (**1**–**5** and **7**–**9**) were previously isolated and elucidated from the authenticated plant material #18332 at the National Center for Natural Products Research (NCNPR). Based on ^{1}H NMR data and HPLC chromatograms, the purity of phytoconstituents **2**–**5** and **7**–**9** was approximately 90–95 % while the purity for compound **1** was 85 % [23]. Compound **6** was purchased from Sigma-Aldrich with 85 % purity. The dried leaves of *M. oleifera* were obtained from Dr. Bello M. Oluwasesan, Nigeria in August 2016. According to National Center for Natural Products Research (NCNPR) guidelines for processing and packaging, the specimen (NCNPR #18332) was identified, prepared, and deposited by Dr. Vijayasankar Raman in the herbarium of NCNPR, School of Pharmacy, University of Mississippi. The morphological and chemical properties were compared with the voucher sample (NCNPR #11 695) at the NCNPR as a process for the authentication. *M. oleifera* supplements' ingredients were identified by searching the websites. Thirteen brands of dietary supplements listed as containing *M. oleifera* were purchased from online sources (▶ **Table 3**). All samples were assigned unique identifiers, and their representative samples were deposited in the botanical repository of NCNPR in the University of Mississippi. The solvents were purchased from Fisher Scientific as analytical grade solvents.

Preparation of standard solutions

Stock solutions containing the standard compounds were prepared separately at a concentration of 1 mg/mL in methanol. Seven different concentration levels were prepared to obtain the calibration curves, and each level was injected in triplicate. The range of the calibration curves was 1–100 µg/mL for the compounds **1**–**4**, **7**, and **9** while 1–500 µg/mL for compounds **5**, **6**, and **8** based on the pilot study using a UHPLC-PDA method (supplementary data).

Sample preparation

Thirteen products were purchased either as a powder or capsule as illustrated in ▶ **Table 3**. For capsules, 3 representative capsules were randomly taken, emptied, and mixed to represent the final product and overcome the sample nonhomogeneity. Plant samples and products were ground to fine powder using Planetary Ball

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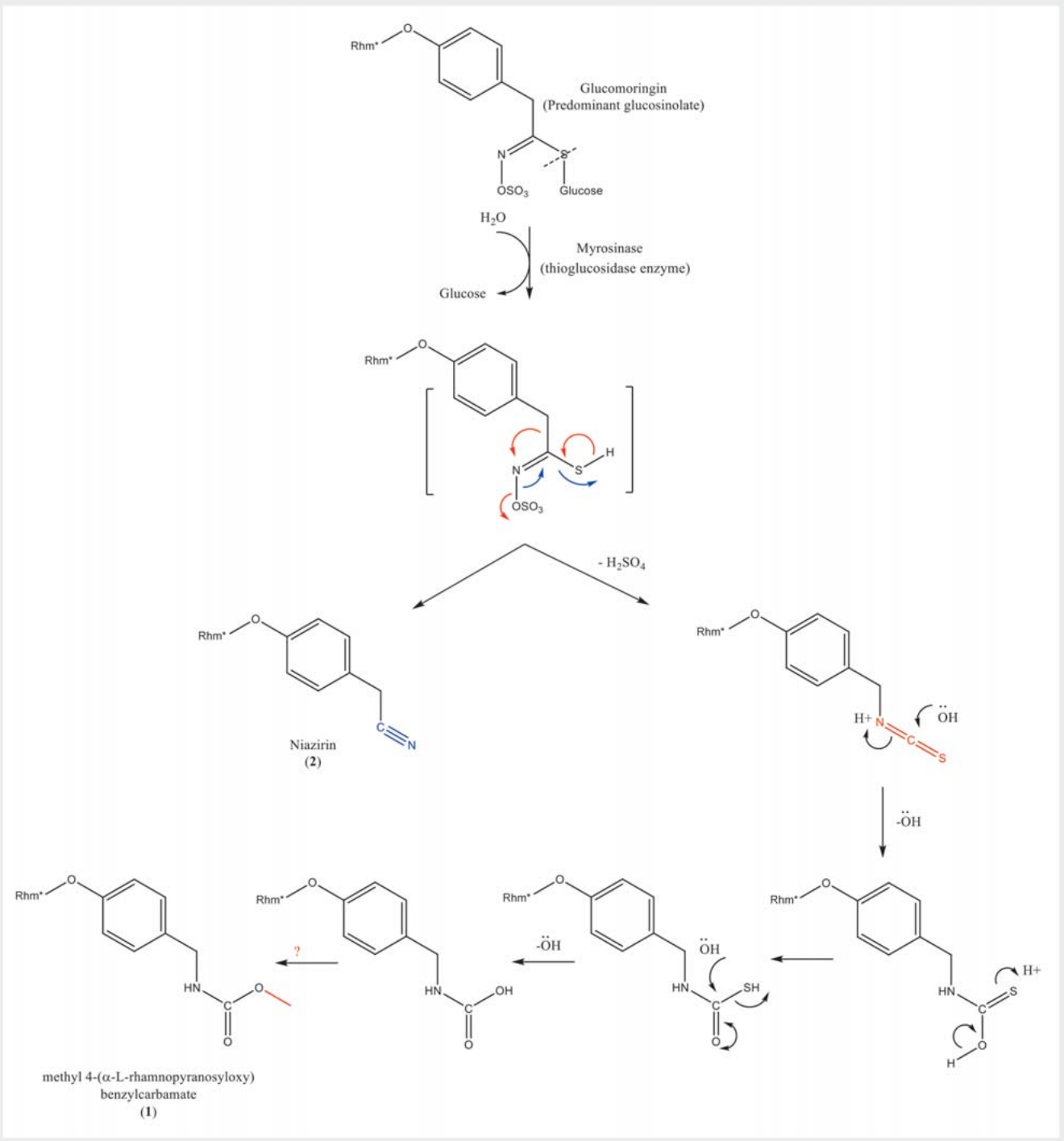

▶ **Fig. 4** Myrosinase-dependent formation of methyl 4-(α-L-rhamnopyranosyloxy) benzyl carbamate (**1**) and niazirin (**2**). Cutting and chewing *M. oleifera* converts the abundant glucosinolate precursor (thioglucoside conjugate) to free glucose and unstable aglycone. Ultimately, biologically active dietary nitrile and isothiocyanate (ITC) can be formed. This process is catalyzed by myrosinase enzyme released from the plant itself or human gut microbiota. * Rhm: α-L-rhamnopyranoside

Mill PM 400 (Retsch). Five hundred milligrams of dried powdered plant samples or products were weighed then sonicated in 2.0 mL of 70% methanol for 30 min followed by centrifugation at 959 *g* for 15 min. Volumetric flask of 10 mL was used to accommodate the supernatant. The procedure was repeated 5 times with 2.0 mL 70% methanol, and the respective supernatants were pooled. The volumetric flask was adjusted to reach 10 mL final volume with 70% methanol then mixed thoroughly. An adequate volume equaling 2 mL was passed through a 0.45 µm polytetrafluoroethylene membrane syringe filter into LC sample vial, and

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▶ **Table 3** Information provided on the label in 13 brands of *M. oleifera* dietary supplements.

NCNPR code #	Dosage form	Serving size	Additional notes	Plant tissue
20737	Powder	0.5–4 Teaspoon	USDA organic	N/A
20738	Powder	N/A	N/A	Leaf
20739	Capsule	3 capsules	1 capsule = 500 mg	leaf
20740	Powder	4 g	USDA organic	leaf
20741	Powder	10 g	USDA organic	leaf
20742	Powder	2 g	N/A	leaf
20743	Powder	1–2 g	USDA organic	leaf
20744	Powder	6 g	USDA organic	leaf
20745	Powder	2 g	USDA organic	leaf
20746	Capsule	0.5 g	N/A	leaf
20747	Capsule	1 capsule	1 capsule = 5000 mg	extract (leaf)
20748	Capsule	2–4 capsules	USDA organic (1 capsule = 500 mg)	leaf
20749	Capsule	0.4 g	N/A	leaf

(N/A) indicates a lack of information

the remaining volume was collected in 10 mL vial and stored in the refrigerator.

Validation procedure

The newly developed UHPLC method was validated regarding linearity, precision, and accuracy according to International Conference on Harmonization guidelines (ICH) [34]. The LOD and LOQ were determined by injecting a series of dilute solutions with known concentrations for each standard. Signal-to-noise ratio equal to 3 and 10 was defined for LOD and LOQ, respectively. The method accuracy was determined in duplicate using a concentration of 10 µg/mL for 2 samples. Three consecutive days with triplicate each served as a test for intra and inter-day variation of the assay.

Instrumentation and chromatographic conditions for UHPLC-PDA-MS analysis

All analysis was carried out on a Waters Acquity UPLC system (Waters Corporation) including the quaternary solvent manager, sampler manager, column heater, PDA, and SQD-ESI-MS detectors connected to Waters Empower 2 data station. An HSS T3, 1.8 µm, 2.1 × 100 mm also from Waters was used. The temperature for the column and sample was maintained at 45 °C and 10 °C, respectively. The mobile phase, at a flow rate of 0.40 mL/min, included water (A) and acetonitrile (B) both containing 0.05 % formic acid applied in the following gradient elution: 0–6 min, 85 % A:15 % B to 72 % A:18 % B; 6–7 min, 72 % A:18 % B to 0 % A:100 % B. Washing procedure for 2 min with 100 % B was applied after separation followed by a re-equilibration period for 4 min. Strong and weak needle wash solutions (90/10 and 10/90; acetonitrile/water) were performed, respectively. The total run time for analysis was 7 min. The injection volume was 2 µL, and the compounds were detected at 220 nm for glucosinolate hydrolysates **1**–**2** and lignans **3**, and **7**–**8**, and 255 nm wavelengths for flavonoids **4**–**6** and **9**. Spiking the samples with standard compounds and then comparing the UV spectra and retention times were necessary for peaks assignment. The mass spectrometric analysis was conducted with a single quadrupole detector (SQD) equipped with an ESI source using the following parameters: capillary voltage 3.0 kV, cone voltage 30 V, source temp 150 °C, desolvation temp 350 °C, and desolvation gas 650 L/h in positive and negative mode. The positive and negative ion modes in the range of *m/z* 100–900 were applied for samples analysis.

Supporting Information

UV, ESI (−) and (+) mass spectra, and calibration curves of compounds **1**–**9** are provided as Supporting Information.

Contributors' Statement

Design of the study: O. I. Fantoukh, Y. H. Wang, Z. Ali, A. G. Chittiboyina, I. A. Khan; Data collection: O. I. Fantoukh, Y. H. Wang, A. Parveen, M. F. Hawwal; Statistical analysis: O. I. Fantoukh, A. Parveen, M. F. Hawwal, G. A. Al-Hamoud; Analysis and interpretation of the data: O. I. Fantoukh, Y. H. Wang, A. Parveen, G. A. Al-Hamoud, Z. Ali, A. G. Chittiboyina, I. A. Khan; Drafting the manuscript: O. I. Fantoukh, M. F. Hawwal, G. A. Al-Hamoud, Z. Ali, A. G. Chittiboyina, I. A. Khan; Critical revision of the manuscript: O. I. Fantoukh, Y. H. Wang, A. Parveen, M. F. Hawwal, G. A. Al-Hamoud, Z. Ali, A. G. Chittiboyina, I. A. Khan.

Acknowledgements

This research is supported in part by "Science-Based Authentication of Dietary Supplements" funded by the Food and Drug Administration [grant number 5U01FD004246-07]. Omer and Mohammed extend their appreciation to the Deanship of Scientific Research and the Research Center, College of Pharmacy, King Saud University for financial support. The authors would also like to thank Dr. Jon F. Parcher for proofreading and correcting grammatical mistakes.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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