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RESEARCH ARTICLE

Isolation and phytochemical characterisation of bioactive compounds from the root extracts of *Momordica dioica* Roxb. ex Willd

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Abstract

Momordica dioica Roxb. belonging to the family of Cucurbitaceae, is a vegetable crop native to India and South Asia. It is widely utilised in traditional medicine for managing diabetes, preventing haemorrhoids and treating a range of other health conditions. Despite its therapeutic and nutritional potential, *M. dioica* remains an underutilised vegetable. In the present study, the root extract of *M. dioica* has been examined in search of various natural components and identified them using various chemical tests and spectroscopic techniques. Dried and finely grounded roots of *M. dioica* were used for the extraction using dichloromethane and methanol as solvent. All the prepared crude root extracts were examined for their preliminary phytochemical testing, confirming the presence of terpenoids, saponins, carbohydrates, glycosides and steroids. The crude extracts were also subjected individually to column chromatography and mainly eight compounds were isolated and identified as bis(11,11-dimethyldodecyl) terephthalate (MD-1), butyl pentanoate (MD-2), tricosanoic acid (MD-3), α-spinasterol (MD-4), hexadecanoic acid (MD-5), stigmas-5-en-3β-ol (MD-6), ursolic acid (MD-7) and β-sitosterol-β-D-galactoside (MD-8). This study reports, for the first time, the presence of MD-1 in *M. dioica.* The identification of various metabolites, including compounds with known therapeutic properties such as, highlights its potential as a promising vegetable for the treatment of several ailments.

Keywords: chromatography; extraction; *Momordica dioica*; phytochemical screening; steroids; ursolic acid

Introduction

Natural products and their derivatives are known as one of the primary sources of lead molecules for treating a wide range of illnesses (1–3). In recent years, the search for alternative therapeutic agents has intensified, predominantly focusing on plant-based metabolites with budding pharmaceutical applications (4–7). Subsequently, pharmaceutical industries have invested substantially in medicinal, clinical and phytochemical research focussed on identifying new bioactive compounds from natural sources, especially secondary metabolites (8).

Momordica dioica Roxb. ex Willd, commonly known as teasle gourd, kakrol, kankro, kartoli, kantoli, kantola or kantroli (9–11), is a perennial, dioecious climbing plant related to the Cucurbitaceae family. It is broadly distributed across India, from the Himalayan foothills to Ceylon and typically grows at elevations up to 1500 m (12). The plant is utilised throughout South Asia as a nutritious vegetable rich in calcium, phosphorus, iron and carotene (13, 14). Conventionally, its leaves, fruits and tuberous roots are used as folk remedies for diabetes (15, 16), while the entire plant has been employed in treating eye infections, fever and various types of poisoning (16).

The tuberous roots of *M. dioica* possess notable medicinal attributes. The root extract exhibits stimulant, astringent and antiseptic properties, while the mucilaginous raw tubers have been reported to show anthelmintic, antifertility, spermicidal and abortifacient activities. Traditionally, the roots are used for treating bleeding piles, bowel irregularities and urinary complications and a powdered form is applied topically to soften the skin and reduce excessive perspiration (17–20). In some practices, the roots are even recommended as a remedy for scorpion stings.

Despite the wide spectrum of traditional uses, the phytochemical composition of the tuberous roots of *M. dioica* remains poorly investigated. Previous studies have primarily focused on the fruits and aerial parts, leaving the root metabolites largely undocumented. Moreover, no comprehensive study exists that combines systematic extraction, chromatographic purification and advanced spectroscopic characterisation of root-derived compounds.

To address this gap, the present study undertakes a detailed phytochemical investigation of *M. dioica* roots. Several metabolites identified in this work are being reported for the first time from this species or even from the genus *Momordica*. Most notably, bis(11,11-dimethyldodecyl) terephthalate (MD-1) is

documented here for the first time from *M. dioica*, establishing a significant novelty of the study. This reinforces the untapped phytochemical potential of the plant's tuberous roots and provides a scientific basis for its traditional medicinal applications.

Materials and Methods

Chemicals

The dichloromethane (DCM) and methanol (CH_3OH) were used for extraction purposes; the column was packed in petroleum ether using silica gel as an adsorbent and eluted with petroleum ether, petroleum ether/ethylacetate, ethylacetate, ethylacetate/methanol gradient in increasing polarity and finally washed with methanol. Pre-coated thin layer chromatography (TLC) plates that were carried out on glass plates coated with 0.2 mm layer of silica gel and on Merck plates were used for chromatographic analyses. The various reagents such as ferric chloride, acetic anhydride, Dragendorff's, dilute sodium hydroxide, concentrated hydrochloric acid, concentrated sulphuric acid, glacial acetic acid and magnesium ribbons were used to establish the nature of phytochemicals in the crude extracts. Deuterated chloroform ($CDCl_3$) and deuterated dimethyl sulfoxide (DMSO-d_6) were used as solvent for spectroscopic investigations. Tetramethylsilane (TMS) is used as an internal standard. The chemicals used in this study were all analytical grades and were purchased from Ranchem, Spectrochem, SDS fine chemicals. This research did not involve human participants or animals.

Equipments

Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were taken on a Perkin Elmer Spectrophotometer using KBr pellets. ^{1}H NMR (300 MHz), ^{13}C NMR (75.47 MHz) and DEPT-135 spectra were recorded on Bruker 300 Spectrometer. Mass spectra were recorded on a Varian MAT 311A instrument by using electron ionisation (EI) at 70 eV and TOF MS on LCT micromass and only the major peaks are quoted. Silica gel of 60–120 mesh from Merck was used for column chromatography.

Sampling procedure

The *M. dioica* plant tuberous roots were selected as the plant material for the present survey based on their standard ethnomedicinal relevance and reported therapeutic properties. Collection and sampling were carried out during the winter season, as this time is known to favour the accretion of secondary metabolites in underground plant parts due to decreased metabolic activity and moisture content. The plant samples were procured from a geographical location (Dargela village, district Kangra, Himachal Pradesh, India; altitude ~3330 ft) to decrease environmental and climatic variability that could impact phytochemical alignment. Healthy, disease-free plants were carefully selected and tuberous roots were physically uprooted to evade mechanical damage. The collected and sorted samples were instantly cleaned with water to eliminate soil and extraneous matter, thereby avoiding contamination during consequent processing.

Shade indoor drying was carried out for 45 days to preserve thermolabile and photosensitive phytoconstituents, as direct sunlight and high temperatures can lead to degradation of bioactive compounds. The dried roots were finely powdered under sterile conditions to ensure uniform particle size, which enhances solvent penetration and extraction efficiency.

Rationale for selection of extraction methodology

Successive solvent extraction technique was employed to get exhaustive extraction of phytochemicals with varying polarities. Dichloromethane (DCM) was initially used to extract non-polar and moderately polar components such as lipids, sterols and terpenoids. This was then followed by extraction with a dichloromethane–methanol mixture (1:1, v/v) to solubilise compounds of intermediate polarity, including certain alkaloids and flavonoids. Finally, methanol was used to extract highly polar constituents such as phenols, glycosides, tannins and saponins.

The use of a mechanical stirrer ensured continuous contact between the plant matrix and solvent, thereby improving mass transfer and extraction efficiency. Each extraction was performed for 48 hr to allow sufficient time for maximum dissolution of bioactive compounds without prolonged exposure that could cause degradation.

Vacuum evaporation was selected for solvent removal to prevent thermal decomposition of heat-sensitive compounds and to obtain solvent-free extracts suitable for phytochemical screening and chromatographic fractionation.

Rationale for preliminary phytochemical screening

Preliminary phytochemical analysis was performed to qualitatively assess the presence of major classes of secondary metabolites such as alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, phenols and glycosides. Standard colorimetric and precipitation-based tests were chosen due to their simplicity, reliability and wide acceptance for rapid screening of crude plant extracts.

These qualitative tests provide an initial chemical profile of the extracts, guiding further chromatographic separation and structural elucidation of bioactive compounds. The use of aqueous solutions of the extracts enabled better interaction with reagents and ensured reproducibility of results.

Detailed extraction of the constituents

Dried and finely ground roots (300 g; obtained from 1 kg fresh roots) were extracted sequentially using DCM, then with 1:1 ratio of DCM:CH_3OH and finally with methanol for 48 hr in succession using a mechanical stirrer. The extracts were filtered and the residual solvent in each extract was individually removed using a vacuum pump to obtain solvent-free extracts: the DCM soluble part (*M. dioica* Extract-1, MDE-1), DCM:CH_3OH (50:50) soluble part (*M. dioica* Extract-2, MDE-2) and CH_3OH soluble part (*M. dioica* Extract-3, MDE-3) respectively. All these fractions (MDE-1 to MDE-3) were further fractionated by using column chromatography. Finally, the percentage (%) yields of the prepared extracts were determined using the following equation (Eqn. 1):

$$\% \text{ Yield} = \frac{\text{Mass of the dried crude extract}}{\text{Mass of the plant materials used for the extraction}} \times 100 \quad \text{(Eqn. 1)}$$

Preliminary phytochemical analysis

The crude extracts MDE-1, MDE-2 and MDE-3 and their aqueous solutions were examined for alkaloids, saponins, tannins, steroids, terpenoids, flavonoids, phenols, etc. by using the methods outlined below (21, 22):

Test for alkaloids

Added Dragendorff's reagent to 5.0 mL aqueous solution of each extract. Red to orange color precipitate inferred the existence of alkaloids.

Test for saponins

A 5.0 mL aqueous solution of each extract was diluted with 5.0 mL distilled water, shaken vigorously. Mix the obtained froth with 2–3 drops of refined oil and again mix strongly. The foam semblance shows the existence of saponins.

Test for tannins

To the 2.0 mL aqueous solution of each extract, add 5.0 mL bromine water. Discoloration of bromine water shows the occurrence of tannins.

Test for steroids

To 5.0 mL of the aqueous solution of each extract, 2.0 mL of chloroform and a few drops of concentrated H_2SO_4 were added and mixed thoroughly. The presence of steroids is confirmed by the appearance of red color in the organic layer.

Test for terpenoids

To 5.0 mL of the aqueous solution of each plant extract, 2.0 mL of chloroform and 2.0 mL of concentrated H_2SO_4 were added. The mixture was heated over a water bath. The appearance of a grey color confirmed the presence of terpenoids.

Test for flavonoids

The Shinoda test was performed to determine the presence of flavonoids in the sample. A 2 to 3 pieces of magnesium ribbons and concentrated HCl were added to 5.0 mL of the aqueous solution of each extract. After 4 to 5 min the appearance of pink color indicated the presence of flavonoids.

Test for glycosides

Liebermann's test was performed to determine the presence of glycosides. To 5.0 mL of the aqueous solution of each extract, 1.0 mL of acetic acid and 1.0 mL of chloroform were added. Then, 1–2 mL of concentrated H_2SO_4 was carefully added along the side wall of the test tube. The appearance of the green color indicated the presence of glycosides.

Test for phenols

A 10 % ferric chloride solution (1.0 mL) was added to 5.0 mL of each plant extract. The appearance of violet color confirmed the presence of phenols. The presence of steroids is confirmed by the appearance of a red color in the organic layer.

Results and Discussion

Extraction yields

The extractions such as MDE-1, MDE-2 and MDE-3 were collected using sequential extraction methodology. The quantities of the dried extracts were 9.3 g, 12.1 g and 5.4 g for DCM soluble extract (MDE-1), DCM:CH_3OH (50:50) soluble extract (MDE-2) and CH_3OH soluble extract (MDE-3), respectively, the percentage yields are reported in Table 1. The resulting crude DCM:CH_3OH (50:50) soluble part (MDE-2) was found to be maximum in yield.

Table 1. Yield (%) of extracted soluble part in each plant's extract

M. dioica extracts	Mass of the dried crude extract (g)	Yield (%)
MDE-1	9.3	3.1
MDE-2	12.1	4.0
MDE-3	5.4	1.8

Preliminary phytochemical test

The extracts MDE-1, MDE-2 and MDE-3 were examined following standard protocols for preliminary phytochemical analysis (Table 2). Phenolic compounds, alkaloids, tannins and flavonoids were absent from all extracts. In contrast, the DCM extract (MDE-1), the DCM:CH_3OH extract (MDE-2) and the CH_3OH extract (MDE-3) all tested positive for steroids, terpenoids and glycosides. Additionally, saponins were detected exclusively in MDE-2 and MDE-3.

The results of this phytochemical screening confirm the presence of key secondary metabolites in the tuberous roots of *M. dioica*, supporting many of its traditional medicinal applications. These findings further suggest that other parts of the plant may also contain valuable bioactive constituents with potential for development into therapeutic agents.

Compound isolation and structure elucidation

Each extract was loaded onto a silica gel-packed column and eluted using a petroleum ether gradient. From MDE-1, a total of 151 fractions (each 140 mL) were collected and subsequently combined into 11 major pooled fractions based on their TLC profiles. Similarly, 55 fractions (each 190 mL) obtained from MDE-2 were merged into 8 pooled fractions, while 70 fractions (each 110 mL) from MDE-3 were consolidated into 5 pooled fractions following TLC analysis (Fig. 1).

These pooled fractions were further purified through repeated column chromatography and recrystallisation, ultimately yielding eight pure compounds (MD-1 to MD-8) (Fig. 2). Structural elucidation of all isolated compounds was performed using UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT and mass spectrometric analyses.

From MDE-1, four compounds were isolated: bis(11,11-dimethyldodecyl) terephthalate (MD-1, 15 mg), butyl pentanoate (MD-2, 25 mg), tricosanoic acid (MD-3, 10 mg) and α-spinasterol (MD-4, 22 mg). From MDE-2, two compounds were obtained: hexadecanoic acid (MD-5, 40 mg) and stigmast-5-en-3β-ol (MD-6, 30 mg). MDE-3 yielded two additional compounds: ursolic acid (MD-7, 60 mg) and β-sitosterol -β-D-galactoside (MD-8, 39 mg).

The compound bis(11,11-dimethyldodecyl) terephthalate (MD-1) is reported for the first time from *M. dioica*. Additionally, MD-2,

Table 2. Preliminary phytochemicals

Crude extracts	Classes of phytochemicals							
	Alkaloids	Saponins	Tannins	Steroids	Terpenoids	Flavonoids	Glycosides	Phenols
MDE-1	–	–	–	+	+	–	+	–
MDE-2	–	+	–	+	+	–	+	–
MDE-3	–	+	–	+	+	–	+	–

(**+**): present; (**–**): absent.

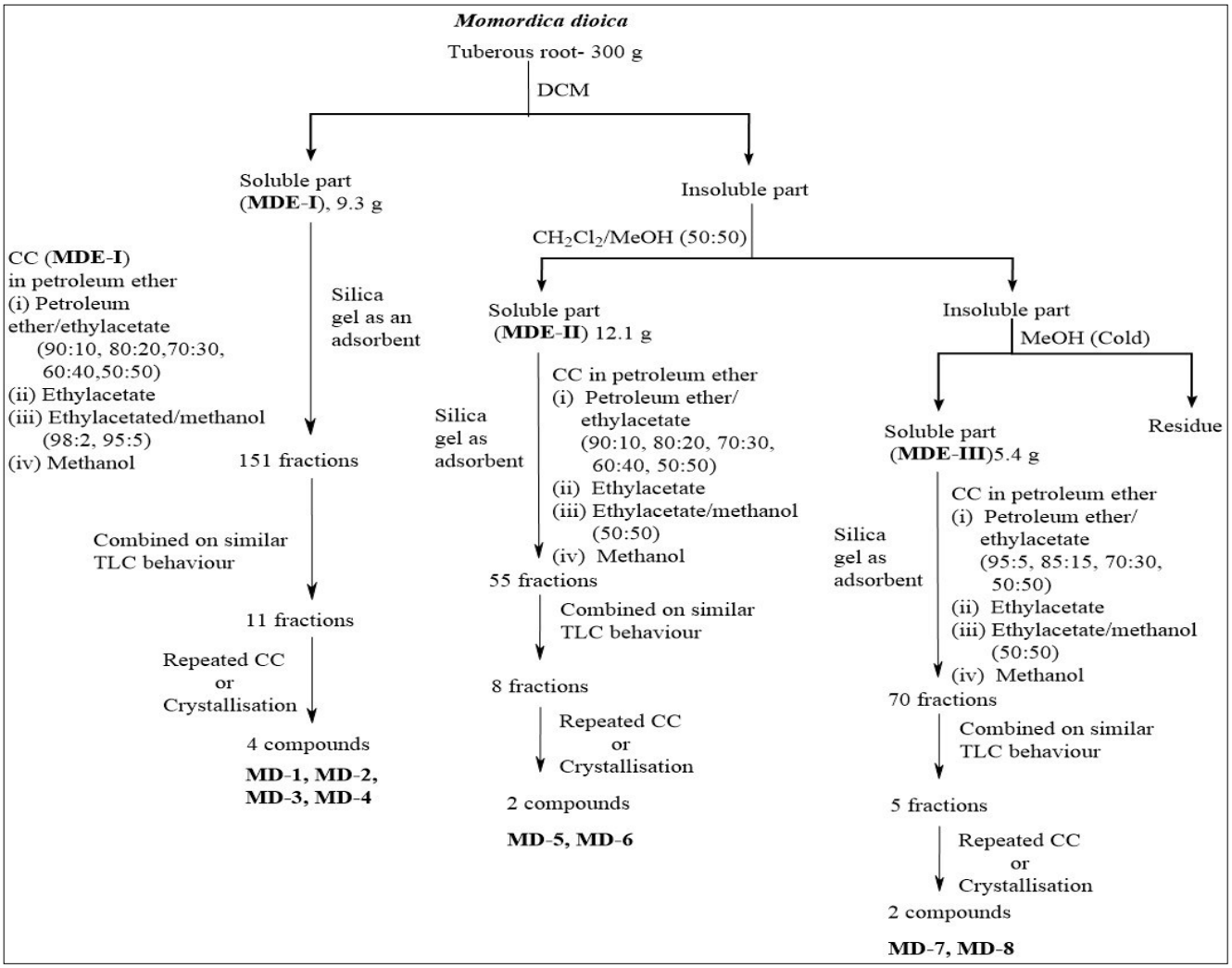

Fig. 1. A schematic illustration of the extraction and fractionation process of *M. dioica.*

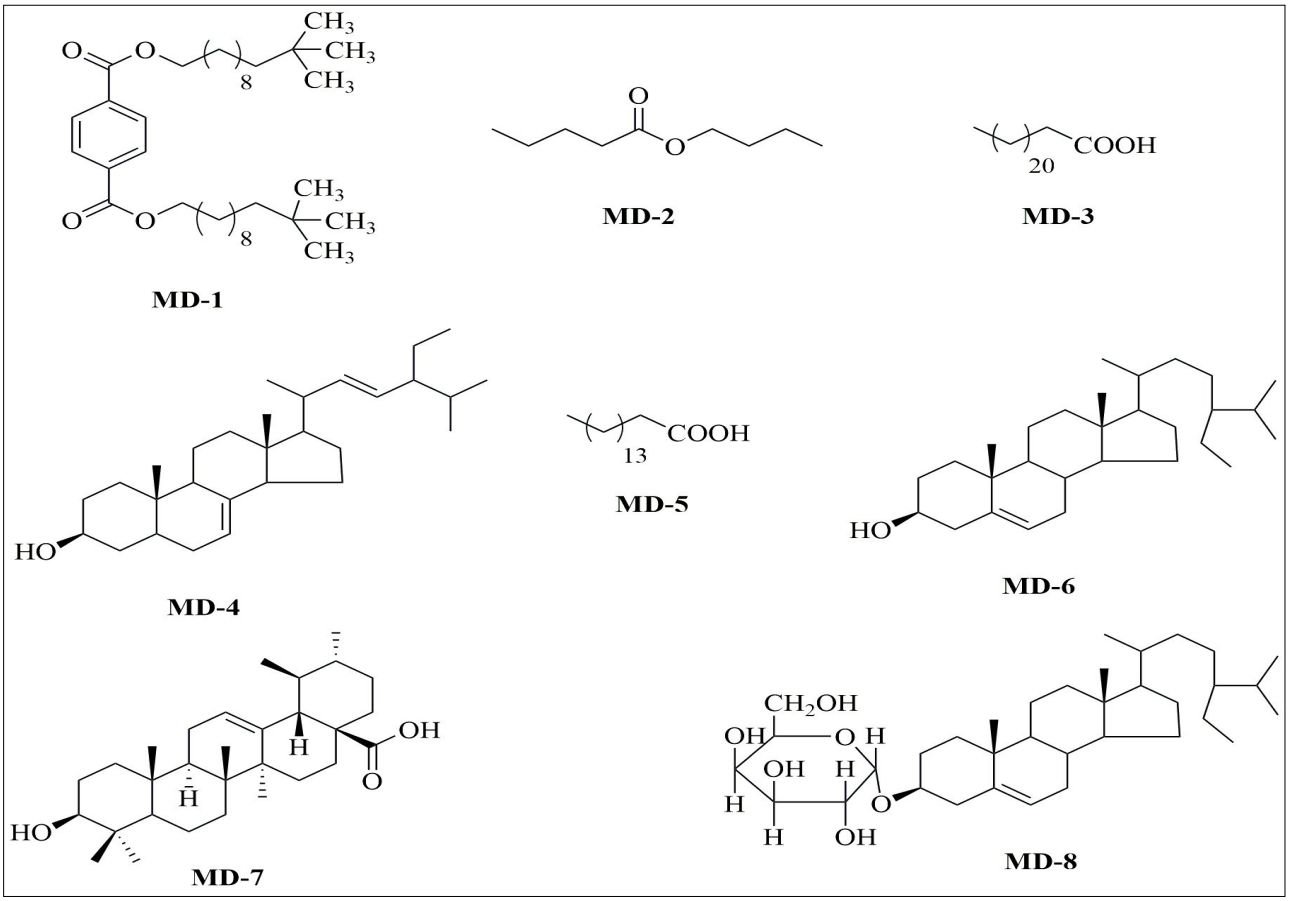

Fig. 2. Structure of compounds MD-1 to MD-8 isolated from the root of *M. dioica.*

MD-3 and MD-8 are reported for the first time from the genus *Momordica*, while MD-5 is newly reported from the species *M. dioica*. These findings highlight the significant phytochemical novelty of the tuberous roots of this plant.

From the authors' perspective, the range of isolated metabolites indicates that the roots of *M. dioica* may serve as a valuable pool of bioactive compounds with potential pharmaceutical applications. The presence of engrained pharmacologically active constituents-such as ursolic acid, β-sitosterol glycosides, long-chain fatty acids and plant sterols-rope in many of the plant's conventional uses, such as application in managing diabetes, inflammatory conditions and gastrointestinal disorders. The identification of the novel compound MD-1 further highlights the unique chemical identity of the roots and suggests that previous studies may have overlooked significant metabolites by focusing predominantly on the fruits and aerial parts.

Current scientific literature on *M. dioica* mainly focuses the nutritional and medicinal properties of the fruits, whereas the roots, in spite of extensive traditional use, have been understated in phytochemical and pharmacological studies. The present survey therefore fills a critical gap by contributing the first systematic chromatographic and spectroscopic characterisation of root-derived metabolites. These data strengthen the potential of *M. dioica* roots as a source of natural compounds of pharmaceutical interest.

Despite the progress achieved, several gaps remain evident:

Lack of biological activity data

Although the isolated compounds include metabolites with known pharmacological relevance, their specific bioactivities within *M. dioica* roots have not been evaluated yet.

Absence of toxicological information

Safety assessment of root extracts is essential to validate their traditional use.

Limited understanding of environmental or seasonal variation

Metabolite composition may vary with geography, climate and plant age, yet such variations remain unexplored and need to be studied.

No comparative phytochemistry between plant parts

Studies comparing roots, fruits, leaves and stems could clarify metabolite distribution patterns and hence yet to be studied.

Unexplored biosynthetic relationships

The synthetic pathways of MD-1 and rare metabolites within the genus remain unknown.

This study offers foundational phytochemical data; however, certain limitations should be recognised:

- Bioactivity assays were not directed hence preventing correlation between metabolites and their pharmacological effects.
- Samples were collected from one geographical location, which may not validate the full chemotypic variability of the species.
- Quantitative analysis (e.g., HPLC, LC-MS/MS) of the isolated compounds was not performed.
- Advanced 2D NMR techniques, although helpful for complex structural assignments, were not widely applied.

These limitations do not weaken the novelty of the findings but highlight areas requiring further investigation in future.

Bioactivity screening and spectral data of the synthesised compounds

To advance the scientific understanding of *M. dioica* roots, the following directions are recommended: Bioactivity screening of isolated compounds for antidiabetic, anticancer, antimicrobial, antioxidant and anti-inflammatory effects. Toxicity evaluations can also be to ensure safe use in traditional medicine and potential pharmaceutical applications. LC–MS/MS, GC–MS or HPLC can be used for quantitative profiling of metabolites using to determine concentration ranges and major constituents (Supplementary data).

Bis(11,11-dimethyldodecyl) terephthalate (MD-1)

Colorless oil (15 mg). R_f = 0.48 in petroleum ether/chloroform (95:5). IR *v* (cm^{-1}): 2929, 2859, 1730, 1600, 1579, 1463, 1379, 1274, 1158, 1039, 742. ^{1}H NMR (δ): 7.72 (doublet, 2H, $J_{coupling\,constant}$ = 7.8 Hz), 7.54 (doublet, 2H, $J_{coupling\,constant}$ = 7.8 Hz), 4.23 (triplet, 4H, 2x -O-CH_2-), 1.66–1.70 (multiplet, 4H, 2x -CH_2CH_2-O), 1.47–1.36 (multiplet, 32H, 2 x -$(CH_2)_8$-), 0.89 (broad singlet, 18H, 6x -CH_3). TOF ES+ m/z (%): 559 (M^++1).

Butylpentanoate (MD-2)

Yellow color solid (25 mg) having melting point 184 °C and literature (23) melting point 185 °C. R_f = 0.45 in petroleum ether/ chloroform (95:5). It gives a light brown color when heated with sulphuric acid. IR *v* (cm^{-1}): 2852, 1738, 1634, 1602, 1515, 1410, 1377, 1270, 1168, 1033, 721. ^{1}H NMR (δ): 4.16 (triplet, 2H, -O-CH_2-), 2.35 (triplet, 2H, -CH_2-C=O-), 1.56 (multiplet, 8H, 2x -$(CH_2)_2CH_2$-C=O & -$(CH_2)_2CH_2$-O), 0.97 (triplet, 6H, 2x-CH_3). TOF ES+ m/z (%): 159 (M^++1, 100).

Tricosanoic acid (MD-3)

White solid (10 mg) having melting point 82 °C and literature (24) melting point 81 °C. R_f = 0.35 in petroleum ether/ethyl acetate (90:10). It gave light brown color on heating with sulphuric acid. IR *v* (cm^{-1}): 3422, 2923, 2558, 1710, 1436, 1377, 1298, 936, 720. ^{1}H NMR (δ): 2.35 (triplet, 2H, $J_{coupling\,constant}$ = 7.5 Hz, -CH_2-C=O). 1.63 (multiplet, 2H, -CH_2CH_2-C=O), 1.26 (broad singlet, 38H, 19 x-CH_2), 0.89 (triplet, 3H, -CH_3), ^{13}C NMR (δ): 179.9, 34.8, 32.0, 31.9, 28.1, 24.7, 22.7, 14.1. TOF ES+ m/z (%): 355 (M^++1, 100).

α-Spinasterol (MD-4)

Colorless needles with a melting point of 117 °C were obtained, whereas the literature reports a melting point of 119 °C (25). R_f = 0.42 in chloroform/methanol (98:2). On heating with 5 % sulphuric acid, an intense violet color obtained. IR *v* (cm^{-1}): 3435, 2923, 2857, 1646, 1462, 1377, 1040, 970, 722. ^{1}H NMR (δ): 5.15 (double doublet, 1H, $J_{coupling\,constant}$ = 8.4 Hz), 5.01(triplet, 1H), 4.98 (double doublet, 1H, $J_{coupling\,constant}$ = 8.1 Hz), 3.61(multiplet, 1H), 1.26-2.02 (multiplet, 29H), 1.04 (doublet, 3H, $J_{coupling\,constant}$ = 6.5 Hz), 0.94 (doublet, 6H, $J_{coupling\,constant}$ = 6.5 Hz), 0.86 (triplet, 3H), 0.79 (singlet, 3H), 0.54 (singlet, 3H) ^{13}C NMR (δ): 139.5, 138.1, 129.5, 117.4, 71.1, 55.8, 55.1, 51.2, 49.4, 43.3, 40.7, 40.2, 39.4, 37.9, 37.1, 31.9, 31.8, 31.4, 29.7, 28.4, 25.4, 23.0, 22.7, 21.5, 20.9, 18.9, 13.0, 12.4, 12.2. DEPT (δ): 139.5 (C-8), 138.1 (C-22), 130.0 (C-23), 117.4 (C-7), 71.1 (C-3), 55.8 (C-17), 55.1 (C-14), 51.6 (C-24), 49.4 (C-9), 43.3 (C-13), 40.7 (C-20), 39.4 (C-12), 37.9 (C-4), 37.1 (C-l), 31.9 (C-25), 31.6 (C-10), 31.7 (C-2), 29.7 (C-6), 28.7 (C-16), 26.5 (C-28), 23.5 (C-15), 22.8 (C-5), 21.7 (C-26), 20.8 (C-11), 20.6 (C-21), 19.7 (C-27), 14.10 (C-19), 13.5 (C-18), 13.0 (C-29). TOF ES+ m/z (%): 413 (M^++1).

Palmitic acid (MD-5)

A white crystalline solid (40 mg) with a melting point of 63 °C was obtained, while the literature reports a melting point of 64 °C (26). R_f = 0.37 in chloroform/methanol (98:02). It gave a light brown color on heating with 5 % sulfuric acid. IR *v* (cm^{-1}): 3432, 2923, 2558, 1710, 1436, 1377, 1298, 936, 720. ^{1}H NMR (δ): 2.34 (triplet, 2H, α-CH_2), 1.66 (multiplet, 2H, β-CH_2), 1.26 (broad singlet, 24H, 12x-CH_2), 0.87 (broad singlet, 3H, CH_3). ^{13}C NMR (δ): 178.2, 35.2, 34.3, 32.0, 31.8, 31.7, 28.1, 25.0, 16.4. TOF ES+ m/z (%): 257 (M^++1).

β-Sitosterol (MD-6)

Colorless needles (30 mg) with a melting point of 134 °C were obtained, while the literature reports a melting point of 136 °C (27). R_f = 0.50 in chloroform/methanol (98:2). Upon heating, it exhibited a violet blue color when treated with 5 % sulfuric acid. IR *v* (cm^{-1}): 3550, 3090, 2965, 1627, 1472, 1369, 1055, 1025, 930, 880, 803 cm^{-1}. ^{1}H NMR (δ): 5.33 (triplet, 1H, $J_{coupling\ constant}$ = 6.9 Hz), 3.22 (multiplet, 1H), 1.34–2.32 (multiplet, 29H), 0.99 (singlet, 3H), 0.89 (doublet, 3H, $J_{coupling\ constant}$ = 6.3 Hz), 0.84 (triplet, 3H, $J_{coupling\ constant}$ = 6.9 Hz), 0.80 (doublet, 6H, $J_{coupling\ constant}$ = 6.9 Hz), 0.69 (singlet, 3H). ^{13}C NMR (δ): 144.1 (C-5), 123.5 (C-6), 74.3 (C-3), 60.1, 57.4, 51.2, 41.5, 41.2, 40.1, 38.6, 37.7, 37.2, 34.5, 33.6, 32.0, 31.4, 30.7, 26.2, 27.8, 24.8, 24.8, 21.2, 22.5, 21.5, 20.2, 19.9, 12.6, 11.6. TOF ES+ m/z (%): 415 (M^++1).

3β-Hydroxy-12-ursen-28-oic acid (MD-7)

Colorless needles (60 mg) with a melting point of 288 °C were obtained, while the literature reports a melting point 290 °C (28). R_f = 0.51 in chloroform/methanol (96:4). IR *v* (cm^{-1}): 3417, 2923, 2853, 1689, 1458, 1368, 1132, 1026, 930, 729. ^{1}H NMR (δ): 5.25 (multiplet, 1H), 3.29 (multiplet, 1H), 2.20 (doublet, 1H, $J_{coupling\ constant}$ 10.2 Hz), 1.71–1.25 (multiplet, -CH_2- & -CH-), 1.18 (singlet, 3H), 0.98 (singlet, 3H), 0.93 (singlet, 3H), 0.86 (doublet, 6H, $J_{coupling\ constant}$ = 6.6 Hz, 29-CH_3 & 30-CH_3), 0.77 (singlet, 6H, 23-CH_3 & 24-CH_3). ^{13}C NMR (δ): 179.1, 139.8, 126.0, 78.4, 57.1, 54.0, 48.2, 48.0, 41.8, 40.2, 40.1, 39.8, 39.4, 37.9, 37.2, 34.1, 32.0, 30.4, 29.3, 29.6, 28.3, 25.5, 24.8, 24.4, 22.6, 19.7, 18.6, 17.4, 16.8. TOF ES+ m/z (%): 457 (M^++1).

Stigmast-5-en-3-O-β-D-galactopyranoside (MD-8)

A white solid (39 mg) with a melting point of 278 °C was obtained, while the literature reports a melting point of 276 °C (29). R_f = 0.49 in chloroform/methanol. It shows a positive Molisch test for carbohydrate & a positive Libermann's Burchard test for triterpenoids. IR *v* (cm^{-1}): 3406, 2943, 2877, 1627, 1462, 1256, 1253, 1108, 1072, 1022, 601. ^{1}H NMR (δ): 5.37 (triplet, 1H, $J_{coupling\ constant}$ = 6.9 Hz), 4.65 (doublet, 1H, $J_{coupling\ constant}$ 3.6 Hz, OH), 4.50 (doublet, 1H, $J_{coupling\ constant}$ = 3.0 Hz, OH), 4.42 (doublet, 1H, $J_{coupling\ constant}$ = 3.6 Hz, OH), 4.39 (doublet, 1H, $J_{coupling\ constant}$ = 7.5 Hz), 3.88 (triplet, 1H, OH), 3.54–3.67 (multiplet, 3H), 3.66 (multiplet, 1H), 3.19–3.37 (multiplet, 3H), 1.01–2.39 (multiplet), 0.90 (singlet, 3H, CH_3), 0.94 (doublet, 3H, $J_{coupling\ constant}$ = 6.8 Hz, CH_3), 0.88 (triplet, 3H, $J_{coupling\ constant}$ = 6.8 Hz, CH_3), 0.81 (doublet, 6H, $J_{coupling\ constant}$ = 6.5 Hz), 0.60 (singlet, 3H). ^{13}C NMR (δ): 140.3, 121.1, 101.9, 77.1, 76.8, 76.6, 73.4, 70.1, 61.2, 56.2, 55.4, 49.6, 45.2, 41.8, 39.2, 38.4, 36.8, 36.2, 35.5, 33.4, 31.4, 29.3, 28.7, 27.7, 25.5, 23.8, 22.6, 20.6, 19.6, 19.0, 18.8, 18.5, 11.7, 11.6. TOF ES+ m/z (%): 414 (M^+-galactose).

Conclusion

The research provides the first comprehensive phytochemical evaluation of the tuberous roots of *M. dioica*, leading to the isolation and characterisation of the various unreported metabolites and thereby significantly expanding the known chemical diversity of this species. The bis(11,11-dimethyldodecyl) terephthalate (MD-1) isolated, is found to be novel natural product, documented for the first time in *M. dioica*. Furthermore, the identification of compounds MD-2, MD-3 and MD-8 is also reported for the first time in the entire genus *Momordica*. The isolation of hexadecanoic acid (MD-5) for the first time in the species further strengthens the novelty of the investigation. Collectively, these results demonstrate that the tuberous roots harbor an array of bioactive constituents not previously associated with *M. dioica*, thereby providing new evidence that supports and scientifically validates its longstanding traditional medicinal uses. The discovery of genus-level and species-level novel compounds highlights the untapped phytochemical potential of the roots and positions *M. dioica* as a promising candidate for future pharmacological exploration, drug discovery and development of phytotherapeutic formulations.

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Authors' contributions

LV and PB carried out sample collection and extraction from dried roots of *Momordica dioica.* AJ and LV planned and designed the experiments; AJ and LV worked on characterisations and identification of compounds. All the authors participated in manuscript compilation and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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