An Effective Method for the Determination of Free-sugar Alcohols and Cyclitols Content in Various Medicinal Plants in the Jordanian Market, Intended for Therapeutic Applications

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In the context of the past and recent global concern about medicinal plants and nutraceuticals for the prevention of various diseases and treatments, this study was conducted on twenty-four samples of medicinal plants, including Saussurea costus, Tribulus terrestris, Bacopa Monnieri, Peganum harmala, Boswellia Carterii, Illicium verum, Artemisia Judaica, and Scutellaria lateriflora, which are used in traditional medicine in the MENA region and other parts of the world. The plant parts obtained were extracted using an updated, efficacious procedure, acetylated, and screened to quantify naturally occurring sugar alcohols (Cyclitols). The analysis was performed using an updated GC-EI/MS method with pulsed splitless injection and cold trapping in SIM mode, and the results were reported in units of $g^{-1}dw^{-1}$. The procedure provides a simple, inexpensive extraction methodology that combines pre-extraction and ether-based removal of oils and other lyophilic substituents, followed by ethanolic maceration and SPE for sugar alcohols from medicinal plants. The studied sugars were found in the following concentration ranges: MI 3.51–39.15 mg g^{-1} dw^{-1}, D-mannitol 0.08–10.51 mg g^{-1} dw^{-1}, DCI 0.09–6.023 mg g^{-1} dw^{-1}, and DPI (ND–0.39 mg g^{-1} dw^{-1}). Tribulus terrestris had the highest MI and DCI content.

Keywords: medicinal plants, myo-inositol, D-chiro-inositol, D-mannitol, D-pinitol, monosaccharides

Introduction

Medicinal plants have been used for thousands of years in traditional medicine worldwide [1-4]. They are among the best natural strategies for enhancing our immune system [2]. In addition, they are captivating due to their diverse biological, therapeutic, and functional properties [3]. Medicinal plants have proven their effectiveness in combating or reducing the severity of several lethal and life-threatening diseases, including cancer, viral infections such as acquired immune deficiency syndrome and hepatitis [5], type 1 and 2 diabetes [6], respiratory diseases [7], and neurodegenerative diseases [8–9]; in addition to polycystic ovary syndrome (PCOS) [10–12]. These properties arise from the presence of diverse secondary metabolites (phytochemicals) and primary metabolites, including sugars and sugar alcohols, which act as signaling molecules to trigger defense responses through signal transduction and pathogen recognition processes [13–17]. Common sugar monosaccharides reported in literature for their widespread occurrence in plants [18], biological functions and properties [19–22], pharmacological interest [23–24] and therapeutic uses [12], in addition to their possible synergic effect including: D-myo-inositol (MI), D-chiro-inositol (DCI), and D-pinitol(3-*o*-methyl-chiro-inositol) (DPI) in addition to D-mannitol [10, 25–42]. Many procedures were adopted and developed for the extraction of sugar alcohols, including cyclitols, in food and plants, including maceration with different solvents and ratios, microwave-assisted extraction, pressurized liquid extraction (PLE), and solid-phase extraction (SPE) followed by GC or LC analysis with different detectors, including the extensively used mass spectrometry (MS) [43–47]. GC–MS has been widely used to analyze low-molecular-weight compounds due to its high resolution, sensitivity, and potential for structural identification and quantitation, particularly for sugars after derivatization [26, 47–48]. In sugar analysis, despite advances in direct chromatographic methods, various derivatization protocols are often used to meet specific analytical needs. Derivatization improves chromatographic separation by altering the physicochemical properties of the derivatives, particularly for complex sugar mixtures. It also enables higher method sensitivity by enabling the use of more sensitive detection modes [49]. Alkylation, silylation, and acylation [50, 52] are derivatization methods to convert polar compounds into volatile and semi-volatile compounds, where an alkyl or acetyl group is added to an organic compound containing active hydrogens (e.g., -OH, -NH, and -SH) and converted

into the corresponding derivative. Acylation can render extremely polar materials, such as sugars, suitable for GC separation [52, 53]. In this research, we aimed to assess and fill the gap for a convenient, robust method for cyclitols and linear sugars from medicinal plants by providing a new modified, efficient, simple, and inexpensive extraction methodology that combines a pre-extraction and removal step for oil and other lyophilic substituents by petroleum ether, followed by ethanolic maceration or extraction for water-soluble sugars and other constituents. This was followed by SPE to remove and clean up other water-soluble constituents that could interfere with the water-soluble sugar analysis, and was finalized with an acetylation procedure to quantify free linear and cyclic sugar alcohols. The novelty of the method lies in providing a refined, direct procedure for the analysis of water-soluble free-sugar alcohols and cyclitols in various plants, including medicinal plants, while accounting for high-oil-content samples, which can complicate extraction and analysis [26, 43–45]. The acetylation procedure used herein is less moisture-sensitive, cheaper, and more convenient to use, as reported previously [52–55], and it also provides a robust and reliable modified GC-EI/MS method that relies on pulsed splitless injection and a cold-trapping technique in SIM mode with three temperature ramps. The modified method provides an effective procedure for removing matrix interferences, especially for oily plants, and delivers high extraction recoveries, short analysis times, simplicity, and sensitivity across low and high concentrations. The combination of procedural steps used in this study has not been published in the literature to date, to our knowledge. Also, filling the gap of absence of literature and knowledge about the presence and the concentrations of sugars MI, DCI, DPI, and D-mannitol that may exist in eight medicinal plant species (*Saussurea costus*, *Tribulus terrestris*, *Bacopa Monnieri*, *Peganum harmala*, *Boswellia Carterii*, *Illicium verum*, *Artemisia Judaica*, and *Scutellaria lateriflora*) collected from herbalist stores in different governorates in Jordan. These sugars, individually or synergically with other phytochemicals, could play vital roles in disease prevention and healing properties in the studied medicinal plants. They are used worldwide in traditional medicine for therapeutic purposes, including memory improvement, anxiolytic effects, insomnia, epilepsy, estrogenic effects, skin diseases, PCOS, neurodegeneration, antidiabetic effects, arthritis, immune stimulation, and other diseases. The data obtained could be important for the nutraceuticals industry, as they point to new sources of the studied sugars.

Materials and methods

Chemicals and reagents

The following sugar standards were purchased from Sigma-Aldrich Company: a standard of myo-inositol 100 g bottle (product no. 15125), a standard of d-mannitol 1000 g bottle (product no. M4125), a standard of d-(+)-chiro-inositol 100 g bottle (product no. 468045), and a standard of d-pinitol 250 mg bottle (product no. H56648). Chemicals used in extraction and cleanup procedures were purchased as follows: Sodium sulfate anhydrous, extra pure (Fluka, Switzerland), and a TELOSA SPE C18 column (Kinesis, Australia). The used solvents are n-Hexane (96%) (Scharlau, Spain), dichloromethane (99.5%) (MACRON, UK), petroleum ether (99.5%) (Sigma-Aldrich, USA), methanol (99.8%) (CARLO ERBA, Italy), and acetyl chloride (98%) (Thermo Scientific, USA). Helium gas was used with a purity of 99.999%.

Instruments and apparatus

Gas chromatography-electron ionization mass spectrometry (GC-EI/MS) on an Agilent 6890 Series II was employed for qualitative and quantitative analysis of the selected sugars. This GC-MS is equipped with an Agilent 7683 N autosampler and a quadrupole mass-selective detector, Agilent 5973 N. A Reacti-Therm heating-stirring module (PEIRCE/18971, USA) was used for the derivatization of sugars. A freeze dryer (BioBase freeze dryer, model number BK-SD10P, China) was used to dry plant extracts from water, while a rotavapor (Buchi R200) was used for volume reduction and evaporation of solvents.

Glassware cleaning

All used glassware was cleaned as reported previously [56]. Briefly, all glassware was first rinsed with solvents such as dichloromethane, hexane, or methanol to remove stains, then washed with detergent and hot water. In the next step, the glassware was rinsed with tap water, then distilled water, and dried in an oven.

Medicinal plant samples

A total of 24 samples from 8 medicinal plant species were collected from local herbalist stores across three governorates in Jordan (Amman, Irbid, and Karak). Each sample (Costus (*Saussurea costus*); *Tribulus terrestris*, Brahmi (*Bacopa Monnieri*), Peganum (*Peganum harmala*), Frankincense (*Boswellia Carterii*), Star Anis (*Illicium verum*), Wormwood (*Artemisia Judaica*), and Skullcap (*Scutellaria lateriflora*) was purchased from three different places in the period from June 2022 to November 2023. All samples, their parts used, and main traditional uses are listed in Table 1.

Sample preparation

A direct, efficacious extraction of water-soluble linear sugar alcohols and cyclitols was performed based on previously published literature with modifications that exclude any hydrolysis steps or reduction steps for the monosaccharides [43, 44, 45, 57, 58, 59], where one gram (1.0 g) of the homogenized dried plant sample was weighed into a round-bottom flask (R.B. Flask) with a magnetic stirrer; 50 mL petroleum ether was added to the round R.B. Flask to remove oils and some other constituents, including pigments from the plant samples; then oils were extracted by

Table 1. Selected medicinal plants used in the present study and their main traditional uses.

Latin name	Parts of plant used	Main traditional uses
Bacopa Monnieri	The leaves and flowers	Memory improvement, insomnia, epilepsy, and as an anxiolytic [72]
Saussurea Costus	The roots	Chronic dastritis, stomach ulcers, rheumatoid arthritis, and inflammation-related diseases [73]
Tribulus Terrestris	The roots and fruit	Kidney stones, polycystic ovary syndrome, low blood pressure, antidiabetic, cardiovascular diseases, gastrointestinal disorders, male virility, and general vitality [74, 75]
Illicium Verum	The fruit	Treat respiratory infections, nausea, sedative properties, expectorant and spasmolytic and estrogenic effects, constipation, and digestive issues [76, 77]
Scutellaria Lateriflora	The leaves, roots, and stems	Nervine effect, allergic cardiometabolic, neurodegeneration, cancer, psychiatric, and infectious disorders [78, 79]
Peganum Harmala	The seeds	Diabetes, rheumatism, treatment of headache and epilepsy [80].
Boswellia Carterii	Sap	Anti-inflammatory, expectorant, antiseptic, diabetes, anxiolytic, and antineurotic effects [81]
Artemisia Judaica	The leaves	Treat gastrointestinal ailments, skin diseases, atherosclerosis, and as an immune-stimulant [82]

stirring for half an hour at 45–50 °C. The ether extract was transferred into a 50 mL polypropylene centrifuge tube and centrifuged at 6000 rpm for 5 min. Then, the clear ether supernatant was discarded, and the pellet was resuspended in 3 mL of ether and transferred into an R.B. Flask. The ether was evaporated to dryness in an oven at 50 °C. Then, sugars were extracted by adding 50 mL ethanol: water (60:40 (% v/v)) and stirring for 2 hours at 40–50 °C. The ethanol content was removed by evaporation at 40 °C using a rotavapor (Buchi R200). The extracted sugar-water fractions were cleaned up by passing them through an activated and preconditioned TELOSA C18 (SPE cartridge) with methanol portions. Active sugar constituents were eluted with HPLC-grade water. Collected water portions for each sample were freeze-dried using a BioBase freeze dryer (BK-SD10P). Ten mg of each lyophilized extract were weighed into a 10 mL screw cap Reacti-Vial (Thermo Fisher Scientific, USA) to be acetylated and diluted to the appropriate volume with hexane and analyzed by GC-EI/MS in the SIM mode with modified method parameters based on established methodologies for monosaccharide composition analysis with some modifications [52, 53, 54, 55].

Preparation of standards

All extracted monosaccharides were acetylated with some modification employing acetyl chloride and sodium acetate as a catalyst, as previously reported [54, 60, 61] where this derivatization step involves chemical modification of the chemical compound functional groups to produce a modified compound with properties suitable for GC analysis (less polarity, higher volatility, higher stability, and higher peak efficiency and detectability [47, 52, 53, 55]. A 10.0 mg portion of each pure standard compound (MI, DCI, DPI, and d-pinitol) was weighed into a 100.0 mL volumetric flask to prepare a 100 mg/L solution. A10 mL portion of the standard mix was freeze-dried to remove water. Then, it was derivatized by transferring the sugar content into a 10 mL reaction vial (Thermo Fisher Scientific, USA) and adding 3 mL of acetyl chloride with excess sodium acetate at 110 °C for 2 hrs. The acetylation reaction was stopped by adding 6 mL of deionized water after cooling the reaction vial under a stream of water. The contents of the reaction vial were transferred into a 50 mL separatory funnel, and the acetylated sugars were extracted in triplicate with 20 mL portions of n-hexane. Collected portions of the hexane were dried from traces of water using the drying agent (sodium sulfate). The volume of n-hexane was reduced to 10 mL using a rotavapor, yielding a standard mixture of sugars at 100 mg/L. Individual standards of 100 mg/L of each sugar were prepared in the same manner. The individual standards and the mix standard with the optimum dilutions were used for the studied sugars with their retention times and mass fragments (m/z) identification in the GC scan mode to build up GC-EI/MS in SIM mode for quantitative analysis and analyte sensitivity and selectivity as demonstrated in the GC chromatograms in the scan and SIM modes in Fig. 1.

GC-EI/MS working conditions

A gas chromatography/mass spectrometry instrument (Agilent 5973N MSD with 6890N GC and 7683 Liquid auto sampler) was used for the chromatographic analysis of sugars using a SCION-5 MS capillary column (30 m × 0.25 mm × 0.25 µm) with helium as the gas carrier. A 2 µL injection volume in pulsed split-less mode for 0.08 min at a constant flow of

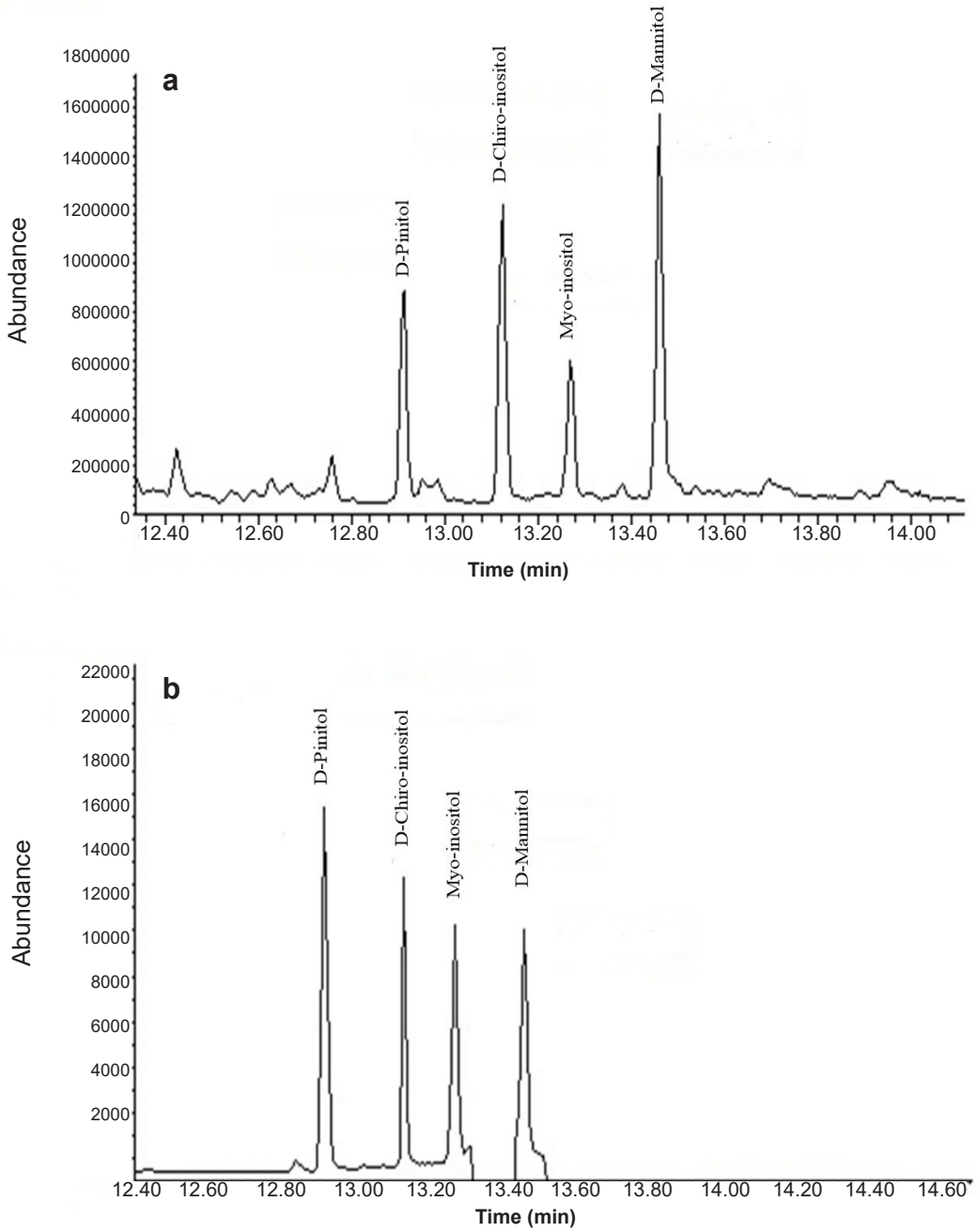

Fig. 1. a – Representative GC chromatogram for a standard mixture (1 mg/L) of sugars in the scan mode; b – Representative GC chromatogram for a standard mixture (320 µg/L) of sugars in the SIM mode.

20 mL/min was used to assess sample concentration, resolution, and sensitivity, with a column flow of 1.7 mL/min. The injector temperature was set at 260 °C, the auxiliary at 290 °C, and the oven temperature program was as follows: 43 °C (hold for 1.50 min), ramp at 24.50 °C/min to 115 °C, ramp at 14.00 °C/min to 210 °C, and ramp at 16.00 °C/min to 290 °C (hold for 5.00 min). The mass spectrometer source was operated in electron-impact ionization (EI) mode at 70 eV and 230 °C, whereas the mass analyzer was operated in SIM mode at 150 °C to improve sensitivity, selectivity, and eliminate matrix effects. The monitored ions and retention times for the four sugars are listed in Table 2.

Method validation

Linearity and range

Calibration curves were established and fulfilled with six concentrations of each standard sugar. A series of aqueous solutions in the range of (0.1–2.0 mg/L) of sugar mixtures, including DPI, d-mannitol, and DCI, in addition to MI in the range (2.0–50.0 mg/L), were prepared. All solutions were lyophilized and derivatized as described in section 2.7. A plot of the area under the curve from the mass detector (SIM mode) vs. concentration was plotted. All calibration curves showed an excellent correlation of ≥ 0.995 as described in Table 3.

Table 2. Retention times and monitored ions (m/z) for the studied sugars (acetylated derivatives).

Sugar	t_R (min) ± 0.05	m/z
D-Pinitol (DPI)	12.87	140, 182, 141, 183
D-Chiro-Inositol (DCI)	13.12	168, 210, 115, 157, 199
Myo-Inositol (MI)	13.26	168, 126, 210, 115, 157
D-Mannitol	13.44	157, 145, 139, 187

Table 3. The LOD, LOQ, and average recoveries with relative standard deviations mix sugar standard.

Relative retention time (Rrt)	Sugars standard	Linear equation	LOD (µg/L)	LOQ (µg/L)	Average recovery ± RSD
1	D-Pinitol (DPI)	y = 534.3x + 3487.2 R^2 = 0.9992	0.24	0.79	97.59 ± 1.77
1.02	D-Chiro-Inositol (DCI)	y =228.75 + 2311.7 R^2= 0.994	0.04	0.15	89.54 ± 0.59
1.03	Myo-Inositol (MI)	Y=361.81x + 854.6 R^2=0.9992	0.07	0.24	101.02 ± 3.78
1.04	D-Mannitol	Y= 334.95x + 33.841 R^2=0.993	0.12	0.39	94.87 ± 2.43

Detection limit and limit of quantitation

Instrumental limit of detection (LOD) was calculated as signal-to-noise (S/N) =3 by decreasing the concentration of the working standard solutions until an S/N ratio of 3 was obtained. The LOQ is determined when the S/N ratio equals 10 or LOQ = 10 S/m = 3 LOD [62]. On the other hand, the limit of quantitation (LOQ) is also the minimum concentration of the substance in the sample that can be measured with acceptable accuracy, repeatability, and precision [63].

Instrument precision

Instrument precision was measured by injecting 3 different concentrations of mix standards of the intended sugars in triplicate: 10, 40, and 80 ppb. The %RSD of the instrumental precision ranged from 0.01 to 0.09 % for all three concentration levels as indicated in Tables S1–S3.

Recovery

The sugar recovery experiment was conducted by preparing three blank samples from medicinal plants. Each blank sample was divided into two halves, each half weighing 1.0 g. The first half was extracted and cleaned up using the aforementioned procedure, then measured by GC-MS. The second half was spiked at concentrations of 2.0, 4.0, and 6.0 µg/g. The recovery tests were done in triplicate. Recoveries were calculated based on the equation below [64].

$$(C_{(blank+spike)} - C_{(blank)})/(C_{(spike\ direct\ into\ GC\text{-}MS)}) \times 100\% \quad \text{Eq.1}$$

All recoveries fell within the trace analysis (80–120 %) acceptable range [65], with %RSD <15 %. [66, 67]. The LOD, LOQ, and average recoveries with percent relative standard deviations (%RSD) are listed in Table 3. The obtained results concerning LOD, LOQ, and extraction recoveries reflect the efficient modified extraction and clean procedure, supported by GC-EI/MS in SIM mode, which provides almost clean GC-MS spectra with resolved peaks for the intended sugars and the absence of any interfering peaks (Fig. S1–S8). The values obtained for LODs, LOQs, %RSD of instrumental precision, and recoveries for the investigated sugars in this study were equivalent to or better than those reported in other publications [26, 43, 47, 48, 57, 58, 59].

Results and discussion

The injections of the acetylated sugar extracts from the studied medicinal plants into the GC-EI/MS SIM mode were accompanied by hexane injection blanks for cross-contamination checks and standard mix standards in the same analysis batch for quality control purposes. Each sample and standard were injected onto the GC-EI/MS column in triplicate. The results of the sugars analysis in the studied medicinal plant extracts are summarized in Table 4.

Comparing the results for the studied medicinal plant samples of the four sugar compounds, as presented in Table 4 and Fig. 2, it was found that MI was present at concentrations ranging from 3.51 mg/g. dw (*Bacopa Monnieri*) to 39.15 mg/g·dw (*Tribulus terrestris*). D-mannitol was found in the concentration range 0.08 mg/g (*Boswellia Carterii*) to 10.51 mg/g·dw (*Artemisia Judaica*). In addition, DCI was found in the concentration range of 0.09 mg/g (*Peganum Harmala*) to 6.23 mg/g·dw (*Tribulus Terrestris*). While DPI was found in the concentration range not detected (ND) (*Artemisia Judaica* and *Illicium Verum*) to 0.39 mg/g. dw (*Saussurea Costus*).

Table 4. The results of the sugars analysis in the studied medicinal plant extracts.

	DPI $\bar{x}$ ± SD (mg/g·dw)	**DCI** $\bar{x}$ ± SD (mg/g·dw)	**MI** $\bar{x}$ ± SD (mg/g·dw)	**D-mannitol** $\bar{x}$ ± SD (mg/g·dw)
Tribulus Terrestris	0.05 ± 0.10	6.23 ± 0.30	39.15 ± 0.44	0.23 ± 0.10
Bacopa Monnieri	0.34 ± 0.10	0.27 ± 0.10	3.51 ± 0.10	1.07 ± 0.10
Peganum Harmala	0.04 ± 0.01	0.09 ± 0.02	23.32 ± 1.92	0.16 ± 0.02
Illicium Verum	0.01 ± 0.01	0.11 ± 0.01	11.08 ± 0.51	2.56 ± 0.11
Artemisia Judaica	ND	0.80 ± 0.26	18.33 ± 1.04	10.51 ± 1.24
Scutellaria Lateriflora	0.28 ± 0.01	0.13 ± 0.03	13.39 ± 0.22	0.68 ± 0.01
Boswellia Carterii	0.05 ± 0.01	0.28 ± 0.16	5.54 ± 0.75	0.08 ± 0.01
Saussurea Costus	0.39 ± 0.06	0.21 ± 0.04	4.24 ± 0.62	0.87 ± 0.14

ND: not detected.

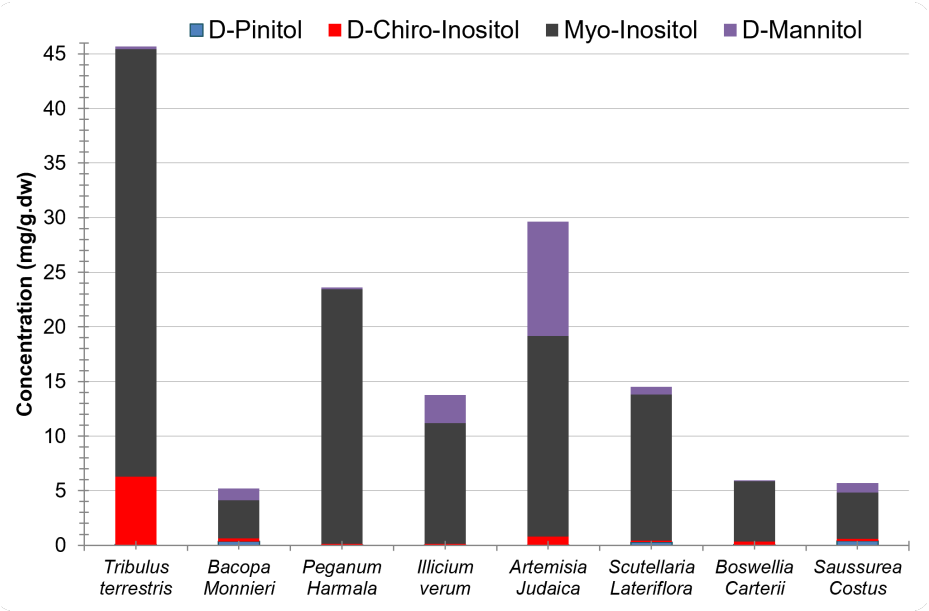

Fig. 2. Concentration of the studied sugars in all studied medicinal plant samples.

In this study, the total concentration of studied sugars in each selected medicinal plant is shown in Fig. 3. It is indicated that the medicinal plant *Tribulus Terrestris* has the highest content of the studied sugars, followed by *Artemisia Judaica.* In addition, the medicinal plants *Saussurea Costus* and *Bacopa Monnieri* exhibit the lowest content of studied sugars.

Each medicinal plant has its own variety of sugars with different concentrations and compositions that may reflect its role as an individual or synergistic therapeutic or preventive bioactive ingredient for illness cases, as well as other biological functions and properties that could be revealed more in the future. Figure 4 shows the levels of each sugar and its diversity in each studied medicinal plant. MI was present at high levels in all studied medicinal plants, followed by d-mannitol and DCI, and then DPI, which was found at the lowest concentrations across all samples.

Significant levels of MI and DCI with traces of other studied sugars were found in the medicinal plant *Tribulus Terrestris*, as shown in Fig. 4. MI was found in varying levels in the other studied medicinal plants with low concentrations of the other sugars. DPI, for example, is present at a concentration of 0.39 mg/g. dw in Saussurea Costus, which is considered to have the highest DPI content among all studied samples. Medicinal plants *Scutellaria Lateriflora*, *Boswellia Carterii*, *Illicium Verum*, and *Bacopa Monnieri* were found to have MI prevalence and low levels of other sugars. In summary, amongst all studied medicinal plant samples, MI was found at an occurrence

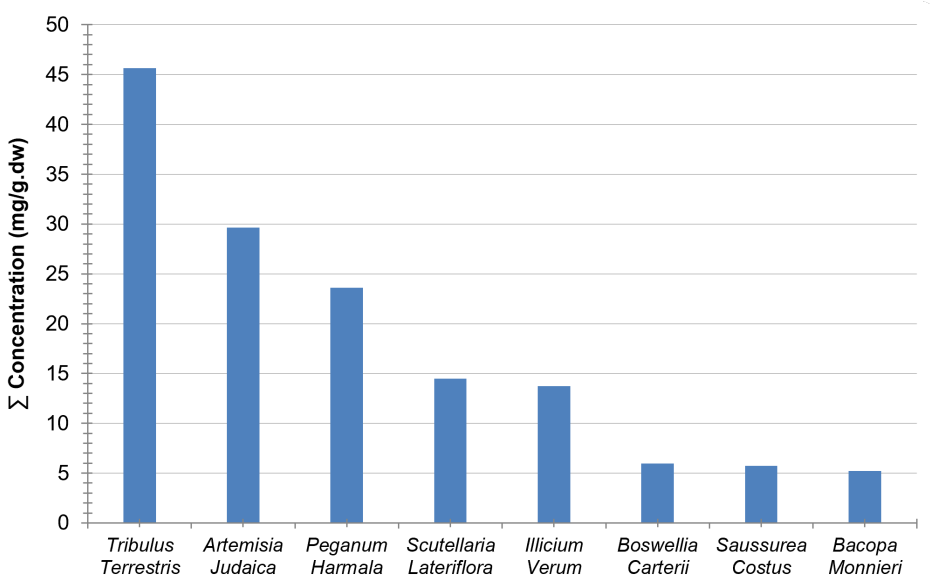

Fig. 3. Total concentration of studied sugars in the studied medicinal plants.

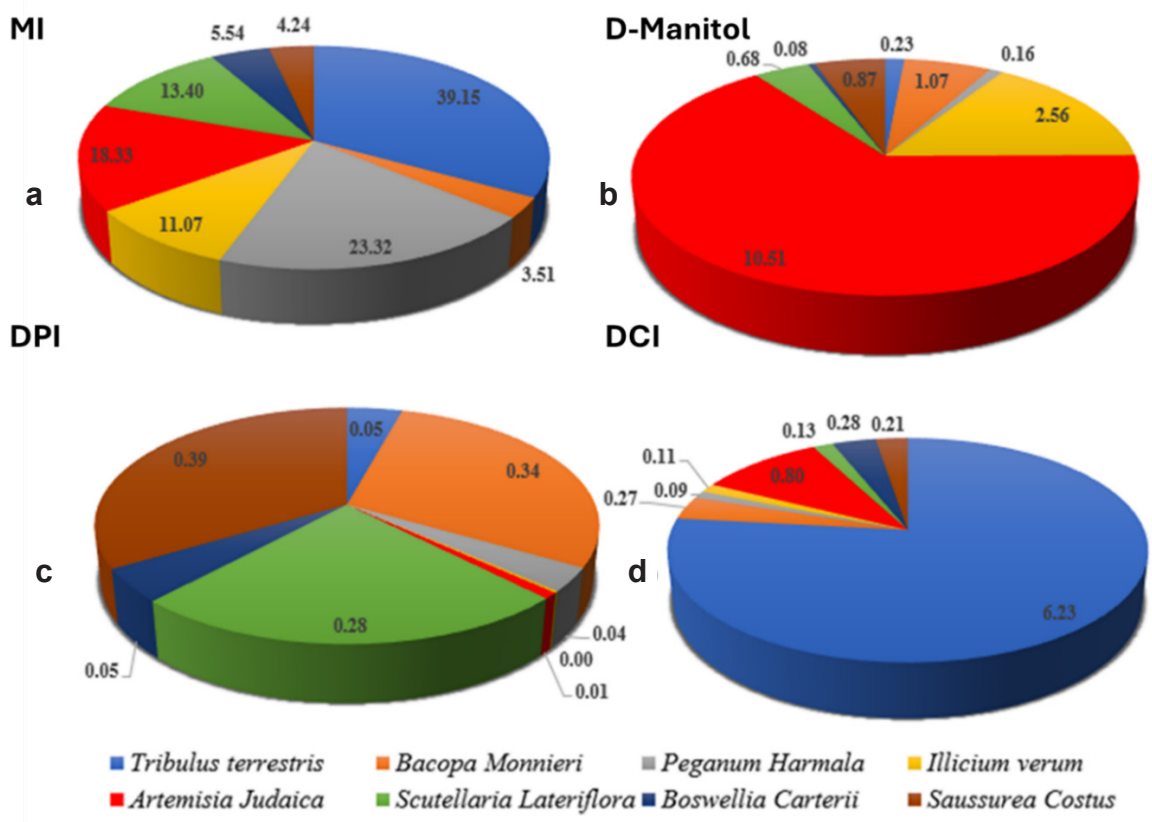

Fig. 4. Representative chart diagram of the distribution of the studied sugars in the studied medicinal plants vs concentration in mg/g·dw: a) MI; b) d-mannitol; c) DPI; d) DCI.

frequency of 82.32%, followed by D-Mannitol, DCI, and DPI with occurrence frequencies of 11.22%, 5.64%, and 0.82%, respectively. The studied sugars were found in the following concentration ranges: MI, 3.51-39.15 mg/g·dw, D-mannitol 0.08- 10.51 mg/g·dw, DCI 0.09- 6.023 mg/g·dw, and DPI (ND-0.39 mg/g·dw). It was found that the highest average sugar concentrations in the medicinal plants were as follows (in mg/g)·dw: *Tribulus Terrestris* (45.67 ± 0.20)> *Artemisia Judaica* (29.67 ± 2.67)> *Peganum Harmala* (23.59 ± 3.70)> *Scutellaria lateriflora* (14.50 ± 0.05)> *Illicium verum* (13.74 ± 0.27)> *Boswellia Carterii* (5.97 ± 0.58)> *Saussurea costus* (5.71 ± 0.41)> *Bacopa Monnieri* (5.20 ± 0.01). This variation

in the occurrence and content of the studied sugars within the medicinal plants could be correlated with their therapeutic purposes. Compared with other studies, a study by Ratiu, I. A., and colleagues (2019) [47] investigating cyclitols and sugars in 40 plant species found that the levels of cyclitols and sugars vary from one plant species to another. Myo-inositol was detected in the concentration range (0.0033-1.2087 mg/g·dw), D-mannitol (0.0633–200.4024 mg/g·dw), D-chiro-inositol (0.0058–1.442 mg/g·dw) and D-pinitol (0.0603– 9.5013 mg/g·dw) [47]. In our study, the concentrations of sugars were found within the following ranges: myo-inositol (3.51–39.15 mg/g·dw), D-mannitol (0.08–10.51 mg/g·dw), D-chiro-inositol (0.09–6.23 mg/g·dw), and D-pinitol (0.003–0.39 mg/g·dw). Another study was conducted by Osamu Negishi and co-workers on several plants, some of which are used in traditional medicine. They found that D-pinitol concentrations lie within a range (260–332 mg/100g fresh weight), myo-inositol (19.2–173.8 mg/100 g fresh weight), and D-chiro-inositol (4.8–191.3 mg/100g fresh weight) [22]. Also, *Withania Somnifera* leaf methanolic extracts were reported to contain up to 73.20 mg/g·dw of myo-inositol [68]. Also, the MI content reported herein for the studied medicinal plants was higher than or approximately equal to that reported in fruits and fruit juices in previously reported studies [44, 69, 70, 71].

Conclusion

Overall summary, this investigative study was performed to provide a simple and inexpensive modified extraction, cleanup, and acetylation procedure, along with a robust modified GC-EI/MS method in SIM mode with pulsed splitless injection, for the simultaneous screening of free cyclic and linear sugars. This modified procedure provides an effective extraction and cleanup process while ensuring high extraction efficiency. Finally, this study determines the presence and levels of certain free inositols and sugars in selected medicinal plants used in Jordan for preventive and therapeutic purposes. The collected medicinal plant species are used for preventive and therapeutic purposes, such as improving memory, lowering blood pressure, treating polycystic ovary syndrome, enhancing male virility, addressing neurodegeneration, treating cancer, and other diseases. MI, DCI, d-mannitol, and DPI were detected in the medicinal plant samples studied. MI, d-mannitol, and DCI were found in appreciable amounts in some of the studied medicinal plants. MI and DCI detected concentrations herein in this study were much higher than previously reported concentrations in some medicinal plants, fruits, and fruit juices, reflecting that these studied medicinal plants are new flourishing sources for MI and DCI. The concentrations of sugars varied from one medicinal plant to another, which could, in part, reflect their medicinal uses. Also, their roles could be associated with other phytochemicals and plant constituents, which are outside the scope of this research. Systematic studies could be conducted to evaluate sugar levels in medicinal plants used in Jordan as traditional medicine, which would enrich knowledge of their therapeutic and preventive uses and provide new natural resources for the nutraceuticals industry. In addition, this study will be the cornerstone for further extensive investigations that screens more free and rare sugars.

Ethics statement

Ethical approval was granted by the ethics committee of the University of Jordan.

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Conflicts of interest

No potential conflict of interest was reported by the author(s).

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Data availability

Data will be made available on request. The data presented in this study are available in the article or in the supplementary material.

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