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Chemical and nutritional profile of Tunisian *Corchorus olitorius* leaves: a valuable, under-exploited leafy vegetable

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ABSTRACT

This study details the chemical and nutritional profile of Tunisian *Corchorus olitorius* leaves. Proximate analysis revealed high levels of dietary fiber (46.4 ± 3.7 g/100 g DM), starch (13.6 ± 1.2 g/100 g DM), and protein (15.4 ± 0.4 g/100 g DM). The protein fraction is rich in essential amino acids, exceeding WHO/FAO/UNU (2007) recommendations for adults, indicating its potential as a plant-based protein source. Despite a low lipid content (3.5 ± 0.4 g/100 g DM), the fatty acid profile is nutritionally balanced, dominated by palmitic, stearic, oleic, linoleic, and linolenic acids. *C. olitorius* also contains a significant ash content (9.3 ± 0.1 g/100 g DM), with high iron (44.9 ± 2.4 mg/100 g DM) and potassium (2348.0 ± 178.5 mg/100 g DM) levels which may contribute to the prevention of deficiencies. The polysaccharides' fraction includes starch, cellulose, pectin, and hemicellulose, suggesting potential for use as natural thickeners or stabilizers in food formulations. LC-MS characterization of secondary metabolites revealed phenolic compounds and flavonoids such as chlorogenic acid, quercetin and kaempferol derivatives, supporting antioxidant potential. These findings highlight *C. olitorius* as a valuable leafy vegetable for the production of high-value nutritional and functional ingredients.

1. Introduction

As global population grows, ensuring food supply and sustainability has become a critical challenge. The State of Food Security and Nutrition in the World (FAO, IFAD, UNICEF, WFP & WHO, 2024) reports that achieving Sustainable Development Goal 2: “Zero Hunger”, remains distant. The global undernourishment rate has stagnated for three years, with 713–757 million people facing hunger in 2023, roughly one in eleven globally and one in five in Africa.

The prevalence of hunger highlights the inadequacy of current food systems in meeting dietary needs. Despite agricultural progress, many still lack access to nutritious food. Rethinking food production and distribution with sustainable solutions is essential to overcome these challenges.

Introducing alternative plant sources, particularly resource-efficient crops, could enhance dietary diversity. Underutilized sources like green leafy vegetables, which are rich in vitamins, minerals, amino acids, and fiber, can contribute to improved nutrition and health outcomes (Kumar et al., 2022; Natesh et al., 2017). In rural areas, these vegetables serve as

an affordable, year-round source of nutrition and are used in traditional medicine to treat various conditions. However, the presence of anti-nutritional factors such as nitrates, oxalates, phytates and tannins in leafy vegetables can interfere with micronutrient absorption, thereby reducing their bioavailability (Natesh et al., 2017). It is therefore important to consider how processing methods such as boiling, cooking, or blanching may help attenuate these effects and improve the overall nutritional value of the vegetables.

Also known as “jute”, “tossa jute”, “mallow of the Jews”, “molokhia” or “mloukhiya”, *Corchorus olitorius* is an annual herbaceous plant, belonging to the Malvaceae family (formerly classified in the Tiliaceae). Widely found in African and Asian countries, this annual plant can reach a height of 2–4 m (Merlier & Montégut, 1982), without any special ecological requirement other than heat. *C. olitorius* grows in various environments and soil types (Sukprakarn et al., 2006).

The genus *Corchorus* includes about 60 species but only *Corchorus capsularis* L. (white jute) and *Corchorus olitorius* L. (red jute) are commonly used for the valorization of natural fibres from the stems (Majumder et al., 2020). In 2021, more than 99 % of the world's jute

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production came from Asian countries, with India and Bangladesh leading the way, according to the Food and Agriculture Organization (FAO, 2024).

While *C. olitorius* is primarily valued for its stem fibers, its leaves are used in traditional recipes and medicine. In West Africa, the Maghreb, and the Middle East, the leaves are consumed in various dishes.

In Tunisia, *C. olitorius*, locally known as “mloukhia”, holds both cultural and economic significance, and is known for its bioactive compounds, minerals, and polysaccharides (Abdallah et al., 2020; Abdel-Razek et al., 2022; Ahmed, 2021b; Ben Yakoub et al., 2018). It is traditionally used to prepare a well-known dish of the same name, which is rooted in Tunisian culinary heritage. The dried and ground leaves are slowly cooked with spices and meat, often as part of festive meals. Beyond its cultural value, *C. olitorius* also represents a source of income for small-scale farmers, where they cultivate, sun-dry, and sell it locally or in national markets.

Beyond its traditional use, the study of *C. olitorius* revealed a wide range of bioactivities, including antioxidant (Nasreen et al., 2022), hepatoprotective (Do et al., 2021; Omeje et al., 2016), anti-inflammatory (Komili et al., 2022; Yan et al., 2013), immune enhancing (Lee et al., 2021), antitumor (Abdel-Wahhab et al., 2015; Taiwo et al., 2016), antimicrobial (M. M. Kim & Park, 2017; Nakaziba, Amana, et al., 2022), antidiabetic (Ahmed, 2021a; Nakaziba, Lubega, et al., 2022), cardioprotective activities (Das et al., 2010), and wound healing properties (Parvin et al., 2015; Yokoyama et al., 2014). Azuma et al. (1999) revealed that this plant contains β -carotene, lutein, and phenolic substances, such as caffeoylquinic acid and quercetin, explaining its antioxidant properties.

Furthermore, the texturizing effect of polysaccharides (PS) extracted from *C. olitorius* was proven, allowing the production of yogurts of good organoleptic quality. The PS, at relatively low concentrations, improved viscosity and prevented sedimentation of dispersed particles (Hussein et al., 2011). This functional property supports the interest in *C. olitorius* not only for its nutritional value but also for its technological potential as a natural texturizing agent in food products. Although few studies detail *C. olitorius* PS composition, dried leaves contain complex PS, cellulose, and hemicellulose, as confirmed by FTIR spectroscopy (Abbas et al., 2023).

Even though *Corchorus olitorius* shows considerable promise, research on its chemical composition remain sparse and geographically limited. Composition data vary depending on origin and methodology, and few detailed studies focus on North African varieties. Given its traditional importance in Tunisia and its underexplored potential, it is relevant to characterize its nutritional and chemical profile in detail.

Therefore, the objective of this study is to provide a comprehensive analysis of the chemical and nutritional composition of *C. olitorius* leaves grown in Tunisia. This work aims to contribute to the valorization of this underutilized plant by highlighting its potential as a local and sustainable food and functional ingredient.

2. Material and methods

2.1. Biomass

The powdered *C. olitorius* leaves used in this study were sourced from a local producer based in Raf Raf (Bizerte governorate, Tunisia). The leaves were sun-dried for a few hours by the producer before being ground with a hammer mill to obtain a particle size in the 100–250 μm range. The resulting powder was then stored at 18 °C in a controlled room until analysis. All analyses were performed using the same batch of powder to ensure consistency. Before each analysis, the moisture content of the samples was determined using a moisture analyzer (MB32, Ohaus) in order to express all results on a dry matter (DM) basis after calculation.

2.2. Proximate analysis

Protein, ash, lipid, and dietary fibre content in *C. olitorius* powder were determined using analysis methods of the Association of Official Analytical Chemists (AOAC, 2012). Total nitrogen (N) was determined using the Kjeldahl method according to AOAC 950.36, with slight modifications. Samples were digested with concentrated sulfuric acid (95–97 %, Supelco®) at 300 °C for 40 min followed by 420 °C for 90 min, until a clear solution was obtained. After digestion, ammonia was distilled into a boric acid solution and titrated with standardized sulfuric acid. Protein content was then calculated using a conversion factor of 4.43 (Yeoh & Wee, 1994). Ash content was determined by incineration at 600 °C according to the AOAC method 942.05 and lipid content was determined using the Soxhlet apparatus with hexane, following AOAC 945.16 method. A total of 180 mL of hexane was used for the extraction, which was carried out for 6 h, allowing as many solvent cycles as possible during this period. Insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) were determined using the total dietary fibre assay Kit from Megazyme (Wicklow, Ireland) with the same procedure as for AOAC method 991.43. Starch content was determined using the K-TSTA Total starch assay Kit from Megazyme (Wicklow, Ireland) following AOAC method 996.11.

Proximate analysis results are presented as mean values of triplicates $\pm$ standard deviation.

2.3. Polysaccharide characterization

2.3.1. Cell wall preparation

According to the method of (Renard, 2005), alcohol-insoluble solids (AIS) were prepared from *C. olitorius* powdered leaves after washing of the powder at room temperature in acetone for 24 h using mechanical agitation. This mixture was filtered by Büchner filtration with a 4 μm pore size, and the residue was boiled in 96 % ethanol (960 mL of ethanol for 1 L of aqueous solution) for 10 min around 85 °C, followed by mechanical agitation overnight at room temperature. This step precedes successive washing of the powder with cold 70 % ethanol (700 mL of ethanol for 1 L of aqueous solution), until the filtrates were sugar-free, as verified by the phenol–sulfuric method of Dubois et al. (1956). Finally, the residue was dried using 96 % ethanol and acetone, and kept overnight in an oven at 40 °C.

Although no enzymatic digestion was performed, starch was largely removed during the AIS preparation process through prolonged boiling in 96 % ethanol and subsequent ethanol washes. However, residual resistant starch may still be present in small amounts.

2.3.2. Extraction of polysaccharides

The PS contained in the AISs were extracted using a reflux heating, with a solid/liquid ratio of 1:20 (g/mL) for 3 h at 100 °C with magnetic stirring (150 rpm). Three different solvents were used: ultrapure water (pH~5.8), aqueous NaOH at 0.002 g/mL (pH 12.7), and aqueous HCl at 4.1 mL per liter of solution (pH 1.3).

After cooling, the mixture was centrifuged (10 000 G, 10 min) and the supernatant containing the PS was collected. The residue was treated with ultrapure water for rinsing and the extraction liquids were combined.

PS present in the supernatant were precipitated with 96 % ethanol for 30 min at room temperature and magnetic stirring of 350 rpm. Büchner filtration of 4 μm was used to recover the precipitate, which was then further rinsed using 96 % ethanol and acetone. The PS fraction was dried overnight at 40 °C.

2.3.3. Neutral sugars composition

Samples must be hydrolysed to obtain sugar residues. Pectin and hemicellulose are degraded into neutral and acidic sugars by a simple acid hydrolysis using diluted sulfuric acid, but cellulose must be solubilized under harsher conditions. Thus, to characterize cellulose, 10 mg

of AIS were submitted to Saeman hydrolysis (Saeman et al., 1954) with sulfuric acid (652 mL/L of concentrated acid in an aqueous solution) for 1 h at room temperature, followed by simple acid hydrolysis to characterize more soluble parts of the cell walls (dilution to 54.5 mL/L of concentrated sulfuric acid, 100 °C for 3 h). Simple hydrolysis on its own was also performed on AIS to estimate cellulose content, by difference. For 10 mg of PS fractions, simple hydrolysis was exclusively used since the PS are already soluble.

Monosaccharides were derivatized to volatile alditol acetates (Englyst et al., 1982) to allow their detection using a GC-2010 Gas chromatography coupled with a flame ionization detector (Shimadzu, Kyoto, Japan).

The column used was an Optima column (30 m × 0.25 mm ID, 0.25 µm) at 220 °C for 20 min, combined with an FID detector at 280 °C. The split ratio was 1:20 and the injection volume 1 µL. The carrier gas was H₂ with a velocity of 50 cm/s.

Quantification was performed by comparison to the internal standard (myo-inositol, purity >99 %). The response factors for each sugar derivative in relation to inositol were calculated using the standard. The results are expressed in anhydrosugars to account for loss of water during the formation of glycoside bonds.

These results are presented as mean values of triplicates ± standard deviation.

2.3.4. Methanol and degree of methylation

Methanol in AISs and polysaccharide fractions was determined by Headspace-GC-MS after saponification, as described by Renard and Ginies (2009). Methyl esters bound to galacturonic acid in pectin were saponified using KOH. Approximately 10 mg of AIS or PS were weighed, and 0.4 mL of CD₃OH (0.6 g/L) was added as an internal standard. Then, 3.8 mL of ultrapure water and 0.8 mL of 1 M KOH were added. The mixture was swirled, and left for 1 h before GC-MS analysis.

The measurement was performed using a GC/MS (5977N, Agilent Technologies, Palo Alto, CA, USA) equipped with a ZB-WAX column (30 m × 0.25 mm ID × 0.5 µm). The PAL Auto sampler system used a gas syringe in head-space mode, injecting 700 µL. The injector temperature was 250 °C with a split ratio of 1:20. Samples were incubated at 40 °C for 10 min with 500 rpm agitation before injection. The isothermal furnace temperature was 40 °C for 6 min, using Helium as the carrier gas at 0.92 mL/min.

Quantification was done with an external calibration containing deuterated and non-deuterated methanol. The amount of methanol in the sample's "head space" was calculated using the peak surface ratio for ion pairs (m/z 31/35), referring to normal and deuterated methanol, over the range of concentration ratios (standards).

The degree of methylation (DM) was calculated as the molar ratio of methanol (MeOH) to anhydrous uronic acids (AUA), and is expressed in % (Eq. (1)):

$$DM(\%) = (MeOH / AUA) \times 100 \quad \text{Eq. 1}$$

Degree of methylation results are presented as mean values of triplicates ± standard deviation.

2.3.5. Uronic acids content

The determination of uronic acids was carried out spectrophotometrically (V-530 Jasco, Tokyo, Japan) according to the 3-phenylphenol test described by Blumenkrantz and Asboe-Hansen (1973). Galacturonic acid solutions were prepared as external standard.

In the case of AISs, samples were prepared after Saeman hydrolysis (Saeman et al., 1954), using the remaining aliquot of the neutral sugars' determination (2.3.3). For PS fractions, the samples are analyzed after saponification, as described in 2.3.4.

0.5 mL of diluted sample or standard were added to 3 mL of sodium tetraborate (0.0125 M in concentrated H₂SO₄), vortexed and heated at 80 °C for 10 min. After cooling, 50 µL of MHDP (3-phenylphenol) (1.5 g/L, in an aqueous NaOH solution of 0.005 g/mL) was added to the

samples or standards and 50 µL of water to the blanks. The absorbance was measured exactly 10 min later.

Results are expressed as mean values of triplicates ± standard deviation.

2.3.6. Macromolecular characteristics of PS fractions

The appropriate mass of samples and standards is mixed with a NaNO₃ solution (0.0085 g/mL of ultrapure water) containing 0.2 g/L sodium azide, to obtain solutions of 3–4 g/L for samples and 0.5 g/L or 3–4 g/L for standards (pectin, dextran and pullulan). These solutions are placed at 4 °C under agitation until the solid is dissolved and then filtered through 0.45 µm (PTFE) before being injected at room temperature.

The equipment of high-performance size exclusion chromatography coupled with multi-angle laser light scattering (HPSEC-MALLS) was used for the determination of molar mass, size and structure distributions by injecting 100 µL of the solutions prepared previously. The HPSEC-MALLS system involved an Ultra-Fast Liquid Chromatography Prominence system (Shimadzu, Kyoto, Japan) including a LC-20AD pump, a DGU-20A5 degasser, a SIL-20A8T autosampler, a CTO-20 AC column oven, a SPD-M20A diode array detector (DAD) and a RID-10A refractive index detector (RID) from Shimadzu (Tokyo, Japan). In addition, a multi-angle laser light scattering (MALLS) detector (DAWN HELEOS 8+ fitted with a K5 flow cell and a GaAs laser, λ = 660 nm), a WyattQELS® system placed at 110° and a ViscostarIII viscometer from Wyatt Technology Corporation (Santa Barbara, CA) were used (Rolland-Sabaté, 2017).

Separations were achieved at 30 °C using three HPSEC columns (PolySep-GFC-P3000, P5000 and P6000 300 × 7.8 mm) and a guard column from Phenomenex (Le Pecq, France). The eluant, a NaNO₃ solution (0.0085 g/mL of ultrapure water) containing 0.2 g/L sodium azide, was filtered through a 0.1 µm, omnipore™ membrane from Millipore (Milford, USA) and degassed before being eluted at 0.6 mL/min.

2.4. Fatty acid profile

2.4.1. Fatty acid methyl esters (FAMES) preparation

Fatty acid methyl esters (FAMES) were prepared from crude lipid extracts of *C. olitorius* obtained by Soxhlet extraction, as described in 2.2, after an acid-catalyzed transmethylation. Around 20 mg of crude lipid extract was added to 1 mL of methanolic sulfuric acid (50 mL/L). 500 µL of Glyceryl triheptadecanoate (C17:0, 2 mg/mL in hexane) was added as the internal standard, and the mixture was heated for 90 min at 85 °C.

After this heating, 1.5 mL of NaCl solution (9 g/L) and 1 mL of *n*-hexane were added, and the mixture was stirred for 30 s. The organic phase was recovered and directly injected for analysis.

2.4.2. GC-FID

Analyses of the organic fraction were performed using an Agilent (Kyoto, Japan) 7820A Gas Chromatograph coupled with a flame ionization detector (FID) according to the AOCS Official Method Ce 1j-07 (AOCS, 2007). The instrument was equipped with a CP-Sil 88 100 m × 0.25 mm × 0.20 µm polar column (Agilent, Tokyo, Japan).

The injection of 1 µL of the samples was performed with a split ratio of 1:20 and the injector temperature was set to 235 °C. The oven temperature was initially 180 °C for 32 min, then increased from 180 °C to 215 °C at a rate of 20 °C/min. The temperature was then maintained at 215 °C for 30 min. The carrier gas was helium with a velocity of 2 mL/min. Calibration curves were established by injecting known dilutions of the standard under the same analytical conditions as the samples, in accordance with the AOCS method.

For peak identification and quantification, a certified reference mixture containing 37 FAMES (Component FAMES Mix37, CRM47885, Supelco®, Sigma-Aldrich) was used. Fatty acid composition (g/100 g of lipidic extract) are expressed as mean values of triplicates ± standard

deviation.

2.5. Protein characterization

2.5.1. Molecular weight distribution

Soluble proteins from *C. olitorius* were extracted using a 0.2 g/L NaOH aqueous solution at 50 °C for 2 h with 150 rpm agitation. After centrifugation at 10 000 G for 10 min, the supernatant was recovered. Protein molecular weight distribution was determined using sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions. Protein samples were mixed with Laemmli buffer (Bio-Rad) containing 2-Mercaptoethanol (50 mL/L) and loaded onto TGX pre-cast gels (Bio-Rad mini-PROTEAN). The gel was run at 45 mA for 1h30, washed with ultrapure water, stained with Coomassie overnight, and destained.

2.5.2. Amino-acid profile

The amino acid composition of *C. olitorius* leaves was determined by IMPROVE (Dury, France) in accordance with ISO 13903:2005 (ISO, 2005) for all amino acids, with the exception of tryptophan. The amino acid composition was determined following acid, oxidative, and alkaline hydrolysis depending on the target residues. For general amino acid profiling, samples were hydrolysed in aqueous hydrochloric acid to cleave peptide bonds and release free amino acids. After hydrolysis, the pH was adjusted, and the volume completed with a loading buffer before filtration to remove particulates. Amino acids were measured by ion chromatography (IC) and separated using an amino acid analyzer with post-column derivatization using ninhydrin. Detection was performed at

$$\text{Carotene} = (1000 \times \text{Abs}_{470} - 0.52 \times \text{Chlorophyll } a - 7.25 \times \text{Chlorophyll } b) / 226$$

Eq. 4

570 nm and 440 nm. An internal standard was used in each analytical batch.

For methionine and cystine analysis, samples were subjected to cold oxidation using hydrogen peroxide and formic acid, followed by acid hydrolysis with aqueous hydrochloric acid. This oxidative pre-treatment converts methionine and cysteine into their stable oxidized forms, preventing degradation during hydrolysis. The post-hydrolysis steps and detection conditions were identical to those used for general amino acid analysis.

Tryptophan was measured by HPLC-FLUO, following alkaline hydrolysis to avoid its degradation under acidic conditions. After hydrolysis, samples were neutralized and filtered. Separation was performed using reversed-phase high-performance liquid chromatography (RP-HPLC), with detection by fluorescence.

2.6. Minerals

Major elements content (P, Ca, Mg and K) and oligo-elements content (Fe, Mn, Cu and Zn) of *C. olitorius* powdered leaves were determined by ICP-OES (Inductively coupled Plasma-Optical Emission Spectrometry). These analyses were conducted by EUROFINIS (Ancenis, France).

For mineral analysis of plant material, an acid extraction using a hydrochloric acid aqueous solution of 100 mL/L in semi-closed digestion reactors was performed. The digestion was carried out at 100 °C for 2 h.

A test portion of 1 g was used, taken from plant material previously dried at 75 °C and ground to a particle size inferior to 1 mm. After digestion, the mineral extract was diluted to a final volume of 50 mL with ultrapure water, filtered, and analyzed by ICP-OES according to the NF EN 11885 norm (AFNOR, 2009).

Each batch of 48 samples included quality controls: a reagent blank to detect potential contamination, an internal reference material

analyzed at both the beginning and end of the sequence to verify accuracy and instrument stability, and duplicate samples to assess repeatability. Additional checks included re-reading the highest calibration standard, with a tolerance of $\pm 5\%$, verification of the blank and dilution stock solution, confirmation of the limit of quantification (first calibration point), and analysis of an independent mid-range control solution.

Results are presented as mean values of triplicates $\pm$ standard deviation.

2.7. Chlorophyll and carotene contents

The chlorophyll and carotene content of powdered *C. olitorius* leaves was determined using a modified method described by Cravotto et al. (2024).

Samples were treated with diethyl ether (1:50 g/mL) and dissolved in an ultrasonic bath for 1 min.

The supernatant was filtered through a 0.4 μm PTFE filter, and its absorbance was measured using a UV spectrophotometer (Libra S6, Biochrom, Cambridge, UK). Maximum absorbance was observed at 660 nm for chlorophyll *a*, 642.5 nm for chlorophyll *b*, and 470 nm for total carotene. Contents were calculated using formulas from Lichtenthaler (1987) (Eq. (2); Eq. (3); Eq. (4)):

$$\text{Chlorophyll } a = 9.93 \times \text{Abs}_{660} - 0.78 \times \text{Abs}_{642} \quad \text{Eq. 2}$$

$$\text{Chlorophyll } b = 17.60 \times \text{Abs}_{642} - 2.81 \times \text{Abs}_{660} \quad \text{Eq. 3}$$

Pigment concentration was expressed as milligrams of pigment per gram of dry matter (mg/g DM) and are presented as mean values of triplicates $\pm$ standard deviation.

2.8. Extracts preparation

Secondary metabolites were extracted from powdered *C. olitorius* leaves using various analytical grade solvents: methanol, ethanol, acetone, ethyl acetate, chloroform, and hexane. The solid/liquid ratio was 1:10 (g/mL) at 50 °C for 1h30 of extraction, with a magnetic agitation of 150 rpm. These extracts were then filtered on PTFE (0.45 μm).

2.9. Antioxidant activity

The antioxidant activity of *C. olitorius* extracts was evaluated using a modified DPPH assay (Brand-Williams et al., 1995) with a FLUOstar Omega microplate reader (BMG LABTECH, Germany). Samples (50 μL) at various concentrations (up to 500 $\mu\text{g/mL}$) or ascorbic acid standard were added to a 96-well microplate, followed by 50 μL of 0.1 mM DPPH solution. After 30 min of dark incubation, absorbance was measured at 520 nm at 25 °C.

The radical scavenging capacity (%) was determined according to Eq. (5) (Blois, 1958):

$$\text{Radical scavenging capacity (\%)} = (\text{Abs}_{\text{control}} - \text{Abs}) / \text{Abs}_{\text{control}} \times 100 \quad \text{Eq. 5}$$

Where $\text{Abs}_{\text{control}}$ is the control absorbance (DPPH solution and MeOH) and Abs is the extract or standard absorbance (DPPH solution and sample or ascorbic acid).

The concentration of extract required to achieve a 50 % reduction in DPPH (IC₅₀) was determined from the curve representing the radical scavenging capacity as a function of extract concentration. IC₅₀ values were determined from linear regression equations, and are expressed as mean values of triplicates $\pm$ standard deviation. Solvent controls were included in the assays to ensure they did not interfere with antioxidant activity.

2.10. Liquid chromatography-mass spectrometry (LC-MS)

For LC-MS analysis, a Waters Synapt G2Si QTOF mass spectrometer was connected to a Waters Acquity I-class plus UPLC. And a Waters HSS T3 column (1.8 μ m, 2.1 $\times$ 100 mm) was used for sample separation. Methanolic extracts of *C. olitorius* were analyzed.

Solvents A and B were formic acid in water and acetonitrile (1 mL/L), respectively. The gradient began with 99 % solvent A for 1 min, increased to 99 % solvent B over 10 min, and was maintained for 2 min. It then decreased to 1 % solvent B over 1.5 min, followed by 1 min of re-equilibration. The flow rate was 0.35 mL/min.

Negative electrospray ionization was employed with a cone voltage of 40 V, source temperature of 100 °C, desolvation gas (N₂) at 800 L/h, cone gas at 50 L/h, and nebulization at 6.5 bar. Data were collected by scanning from 50 to 1500 m/z. Prior to each analytical sequence, the mass spectrometer underwent a two step calibration process. First, leucine enkephalin was used to automatically adjust and verify the detector's electronic parameters based on signal quality. Then, a sodium formate solution was used to perform a mass calibration across the full m/z range. During all sample acquisitions, leucine enkephalin was continuously infused as a lock mass to correct for instrumental drift and ensure accurate and stable m/z measurements throughout the run.

Phytochemical compounds were identified using manual interpretation and SIRIUS software (v.6.0.0). The elution order, mass-to-charge ratio (m/z), and fragmentation patterns were compared with literature data. MS₂ spectra were processed using MZMine and SIRIUS, with molecular formula computation at 20 ppm mass accuracy and elemental composition restricted to C, H, and O. MS₂ spectra were also compared with Mass Bank Europe databank.

2.11. Statistical analysis

PS characterization results reproducibility is expressed as pooled standard deviation (Pooled SD). Pooled SD was calculated for each series of replicates using the sum of individual variances weighted by the individual degrees of freedom (Box et al., 1978).

Data calculations, visualization, and statistical analyses were performed using Microsoft Excel. The data were subjected to a one-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$) for multiple comparisons of means. Different letters indicate statistically significant differences between solvents ($p < 0.05$). The analysis was performed in Excel using the Data Analysis Toolpak, and letters were assigned based on the results of Tukey's test.

3. Results/Discussion

3.1. Proximate analysis

The powder of *C. olitorius* leaves showed an average moisture content of approximately 5–6 g/100 g, depending on ambient weather conditions. All analyses were therefore conducted on dry material, and after collecting moisture content values, the results expressed on a dry matter basis. The proportion of total sugars in *C. olitorius* is usually quantified using the method of DuBois et al. (1956) (Ben Yakoub et al., 2020; Biswas et al., 2022; Idiris et al., 2010). Unfortunately, Dubois method does not allow the differentiation between simple sugars and hydrolysed biopolymers (cellulose, starch ...). In addition, these complex PSs may not be fully hydrolysed. Thus, an analysis of starch, soluble and

Table 1

Proximate analysis of Tunisian *C. olitorius* powdered leaves (g/100 g of dry matter), n = 3.

	<i>C. olitorius</i>
Protein	15.37 $\pm$ 0.38
Lipid	3.46 $\pm$ 0.36
Ash	9.34 $\pm$ 0.13
Insoluble dietary fibre	35.53 $\pm$ 1.62
Soluble dietary fibre	10.90 $\pm$ 3.35
Starch	13.57 $\pm$ 1.22
Others	11.83 $\pm$ 3.95

insoluble dietary fibre allows a better description of *C. olitorius* leaves composition.

IDF represents PS that are not soluble throughout the digestive tract such as hemicellulose, cellulose, and lignin, but also resistant starch. On the other hand, SDF regroups PS that can solubilize in water, such as "traditional" soluble fibre and oligosaccharides (Lunn & Buttriss, 2007).

Polysaccharide biopolymers (starch, SDF, and IDF) account for 60 g/100 g of *C. olitorius*, highlighting the nutritional value of its leaves (Table 1). The starch content is notably high at 13.57 $\pm$ 1.22 g/100 g of dry matter, unusual for leaves where starch is typically temporary. At night, leaf starch is hydrolysed to maltose and glucose to support sucrose synthesis, respiration, and growth (Zeeman et al., 2007).

Some Malvaceae species, like *Theobroma*, have starch grains in their leaves (Garcia et al., 2014). Anatomical studies of other Malvaceae, such as *Herissantia crista*, revealed starch-storing parenchyma and aerenchyma in the stem pith (Silva et al., 2023). This supports the idea that starch presence is not unusual in the Malvaceae family and that their leaves may store starch.

According to the IDF and SDF values (respectively 35.53 $\pm$ 1.62 and 10.90 $\pm$ 3.35 g/100 g of dry matter), it is possible to hypothesize that cellulose, hemicellulose, and other PS are present in the leaves as previously stated by Abbas et al. (2023). This might explain why *C. olitorius* powdered leaves present an interesting texturing effect upon contact with water, becoming a viscous mixture.

C. olitorius leaves contain a high amount of protein (15.37 $\pm$ 0.38 g/100 g of dry matter), which may contribute to the emulsifying properties of its extracts (D.-Y. D. Kim & Kim, 2022), as proteins can act as emulsifiers (McClements, 2004). The ash content is 9.34 $\pm$ 0.13 g/100 g of dry matter, indicating high mineral levels. Lipid content is low at 3.46 $\pm$ 0.36 g/100 g, aligning with findings by Guil-Guerrero and Rodríguez-García (1999) for edible wild plants (1.46–3.81 g/100 g). The remaining unquantified fraction (11.83 g/100 g) was calculated by subtracting the total of all quantified components from 100 g. Although this value is hypothetical, it may include primary or secondary metabolites such as chlorophylls, carotenoids, and other non-identified compounds.

3.2. Polysaccharides

3.2.1. Monosaccharide profiles

The analysis of AIS from *C. olitorius* leaves indicates that the PSs present would be cellulose (350 mg/g of cellulosic glucose), but also pectin (177 mg/g of uronic acid and 15 % DM) and residual starch (372 mg/g of glucose) (Table 2). It is however sensible to keep in mind that the glucose patterns could be constitutive of starch but also of hemicellulose and other complex PS. The presence of other neutral sugars (arabinose, xylose, galactose, and mannose) could be attributed to the ramifications of pectin (rhamnogalacturonan I and/or II) but could also be related to the presence of different complex hemicelluloses.

No previous monosaccharide profile has been reported directly from the powdered leaves of *C. olitorius* or its AIS. Existing studies focus on extracted polysaccharides (PSs), typically using aqueous extraction. Compared to literature (Table 3), water-extracted PSs from Tunisian *C. olitorius* show high glucose content (616 mg/g), possibly due to starch

Table 2

Yields (mg/g of dry matter from AIS), composition (mg/g), and macromolecular characteristics (HPSEC) of AIS isolated from powdered Tunisian *C. olitorius* leaves, and PS fractions extracted from AIS, n = 3.

Sample	Yield	Rha	Ara	Xyl	Man	Gal	Glc	Cel. Glc	AUA	MeOH	DM (%)	Mw (x10 ³ g mol ⁻¹)	Mn (x10 ³ g mol ⁻¹)	Polydispersity (Mw/Mn)	Intrinsic viscosity [η]z (mL/g)
AIS	–	16	43	53	8	25	372	350	177	4	15	–	–	–	–
Acid PS	29	19	5	13	0	25	719	–	164	9	38	48	13	4	133
Neutral PS	20	21	25	14	0	25	616	–	137	4	20	1015	324	3	560
Alkaline PS	16	37	82	37	0	44	202	–	96	<0,5	<3	627	61	10	1359
Pooled SD	–	2	0	3	4	1	2	28	66	0	3	–	–	–	–

AIS: alcohol-insoluble solid, PS: polysaccharides. Pooled SD: pooled standard deviation. Rha: rhamnose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Cel. Glc: cellulosic glucose, AUA: anhydrous uronic acids, MeOH: methanol, DM: degree of methylation. Mw: Molecular weight, Mn: Molecular number.

Table 3

Literature review of extraction methods and monosaccharide composition (mg/g) of extracted PS from *C. olitorius* leaves.

PS preparation	Rha	Ara	Xyl	Man	Gal	Glc	GalA	GluA	Fuc	Reference
H ₂ O extraction and precipitation EtOH	238	108	73	–	140	78	188	155	21	Oh and Kim (2022)
H ₂ O extraction, dialysis, precipitation EtOH	220	150	–	100	230	180	–	20	–	Ben Yakoub et al. (2020)
H ₂ O extraction, acetone precipitation, dialysis, precipitation EtOH	102	0	0	0	39	82.5	166	358	67	El-Mahdy and El-Sebaiy (1984)

PS: polysaccharides. Rha: rhamnose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, GalA: galacturonic acid, GluA: glucuronic acid, Fuc: Fucose.

and cellulose. Monosaccharide composition varies widely, possibly influenced by growing conditions (Kubacka-Zębalska & Kacperska, 1999), seasonality (Dungait et al., 2010), and plant variety.

Water-extracted PS have a monosaccharide composition similar to AIS (Table 2). Acid extraction (HCl in aqueous solution) mainly extracts glucose (likely starch since cellulose is more resistant to acidic pH) and pectin. On the contrary, an alkaline extraction (NaOH in aqueous solution) extracts less glucose and uronic acids, but liberates the neutral sugars in larger quantity, indicating the extraction of hemicellulose.

3.2.2. Macromolecular characteristics

Extraction conditions affect PS composition and yield. The pH of extraction influences greatly the molecular weight (Mw) of the PS obtained: an extraction at neutral pH enhances the extraction of high-molecular weight polymers (Mw = 1015 × 10³ g mol⁻¹) with low polydispersity. The polydispersity is a measure of the distribution of molecular weights in a polymer sample, and is calculated as the ratio of weight-average molecular weight (Mw) to number-average molecular weight (Mn). The higher the polydispersity, the wider the range of molecular weights for a sample.

An alkaline extraction liberates polymers with a high polydispersity (Mw/Mn = 10), therefore both high and low molecular weight structures are extracted, making an average molecular weight of 627 × 10³ g mol⁻¹. Finally, an acidic extraction extracts mostly molecules with a low molecular weight (Mw = 48 × 10³ g mol⁻¹, Mw/Mn = 4).

Intrinsic viscosity of PSs increases with extraction pH, reflecting structural changes. Acidic extraction may yield pectin-rich PSs (mainly as short chains), with low viscosity, while alkaline extraction may yield hemicelluloses with high viscosity.

3.3. Lipids

Palmitic (C16:0), stearic (C18:0), oleic (C18:1 cis), linoleic (C18:2 cis) and linolenic (C18:3 α) acids account for 94.94 g/100 g of the lipid fraction in the leaves of *C. olitorius* (Table 4).

These fatty acids were expected, as the structural neutral lipids of all plant cells are almost exclusively 16 and 18-carbon fatty acids, with up to three double bonds (Millar et al., 2000). Linoleic acid (C18:2 cis) remains the most present fatty acid in the lipid extract of *C. olitorius*. This extract is also rich in linolenic acid (C18:3 α) with 10.4 g/100 g of the lipid fraction. Linolenic acid is an ω-3 fatty acid known to have health benefits and plays an important role in a lot of physiological functions

Table 4

Fatty acid composition (g/100 g of lipidic extract) of *C. olitorius* crude lipid fraction after GC-FID analysis of the corresponding FAMES, n = 3.

Identified FAMES	Fatty acid composition
C6:0	0.24 ± 0.01
C8:0	0.53 ± 0.07
C13:0	0.29 ± 0.24
C14:0	0.30 ± 0.01
C15:0	0.36 ± 0.01
C16:0	18.07 ± 0.08
C16:1	0.21 ± 0.01
C17:0	0.78 ± 0.04
C18:0	4.71 ± 0.02
C18:1 cis	20.36 ± 0.04
C18:2 cis	41.40 ± 0.34
C18:3 α	10.40 ± 0.18
C20:0	0.52 ± 0.01
C20:2 cis	0.21 ± 0.15
C20:3 (8,11,14) cis	0.68 ± 0.02
ΣSFAs	25.81 ± 0.27
ΣMUFAs	20.57 ± 0.05
ΣPUFAs	52.70 ± 0.42
ω-6/ω-3 ratio	4.07 ± 0.04

SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, PUFAs: poly-unsaturated fatty acids.

(Stark et al., 2008). According to Abbas Saleh and Nguyen (2016), *C. olitorius* leaves are mainly composed of linolenic acid (C18:3 α), up to 40 g/100 g of the total fatty acids. It is possible to assume that the variation of the growing site affects the lipid composition of *C. olitorius* leaves.

Interestingly, a small amount of C17:0 (0.78 ± 0.04 g/100 g lipid extract) was detected in the crude lipid fraction. While C17:0 is commonly used as an internal standard in fatty acid analysis and is typically absent or only present in trace amounts in plant matrices, its occurrence here may reflect either minor contamination possibly of bacterial or fungal origin, or low levels naturally present in the plant.

Within the Malvaceae family, *Malvaviscus arboreus* has been shown to contain unusual odd-chain fatty acids, including 9-heptadecynoic acid, in its leaf lipid profile (Abdelhazef et al., 2020), supporting the plausibility of such compounds being present in *C. olitorius* leaves as well.

Western diets, high in ω-6 fatty acids, increase the risk of inflammatory diseases and obesity (Gonzalez-Becerra et al., 2023). The World

Health Organization (WHO, 2023) recommends a maximum ω -6/ ω -3 ratio of 5 for lipid intake. *C. olitorius* has an average ratio of 4, making its leaf oil a nutritionally beneficial lipid source.

3.4. Proteins

As determined with the proximate analysis, *C. olitorius* is a plant relatively rich in protein.

Two protein sizes were determined after electrophoresis, with molecular weight of 48 and 12 kDa (Fig. 1). As described by Maity et al. (2009), 42 polypeptides bands were identified in *C. olitorius*, with strong bands around 47 kDa, 36.4 kDa and 20.5 kDa concurring partially with the results presented. It is necessary to keep in mind that drying, grinding, storing, extraction and purification techniques can alter proteins, potentially explaining these differences in size.

Additionally, to their size, the composition of the protein fraction is especially important for the nutritional profile of *C. olitorius* (Table 5).

In this study, glutamic acid (GA) and aspartic acid (Asp) were the most abundant amino acids in *C. olitorius*, at 2.99 and 1.80 g/100 g, respectively. Such high levels are consistent with previous reports on leafy vegetables, where GA and Asp are typically dominant. In spinach (*Spinacia oleracea* L.), GA and Asp accounted for approximately 12 % and 11 % of the total amino acid content, respectively (Lisiewska et al., 2011).

GA, though classified as non-essential, is crucial for protein solubility and structure (Uversky, 2013). As for Asp, it exists in two isoforms: L-aspartic acid (L-Asp), the main form and D-aspartic acid (D-Asp). L-Asp is crucial, for urea synthesis and neurotransmission, and D-Asp supports brain development and hypothalamus regulation (Holeček, 2023). *C. olitorius* is also rich in Proline (1.61 g/100 g) and other essential amino acids (EAA) like Leucine (1.60 g/100 g) and Valine (1.10 g/100 g). An EAA is an amino acid that the body cannot produce on its own (or in sufficient amounts), making it necessary to obtain through food to support proper bodily functions.

Phenylalanine (Phe) is an EAA, while Tyrosine, derived from Phe, relies on its supply. Similarly, Methionine (Met) is essential, but

Table 5

Amino acid content (g/100 g of dry matter) from powdered Tunisian *C. olitorius* leaves, n = 1.

Amino acids	<i>C. olitorius</i>
Alanine (Ala)	1.04
Arginine (Arg)	1.05
Aspartic acid (Asp)	1.80
Cysteine (Cys)	0.36
Glutamic acid (Glu)	2.99
Glycine (Gly)	0.96
Histidine (His)	0.46
Isoleucine (Ile)	0.84
Leucine (Leu)	1.60
Lysine (Lys)	1.07
Methionine (Met)	0.30
Phenylalanine (Phe)	1.05
Proline (Pro)	1.61
Serine (Ser)	0.85
Threonine (Thr)	0.83
Tyrosine (Tyr)	0.71
Valine (Val)	1.10
Tryptophan (Trp)	0.36

Table 6

Amino acids content from powdered Tunisian *C. olitorius* leaves and indispensable amino acid requirement values (g/100 g of proteins) (WHO/FAO/UNU, 2007).

Indispensable amino acids	<i>C. olitorius</i>	WHO/FAO/UNU (2007)
SAA	3.48	2.2
AAA	9.27	3.0
Tryptophan	1.90	0.6
Isoleucine	4.43	3.0
Leucine	8.43	5.9
Lysine	5.64	4.5
Threonine	4.37	2.3
Valine	5.80	3.9
Histidine	2.42	1.5

SAA: sulphur amino acids (Methionine + Cysteine), AAA: aromatic amino acids (Phenylalanine + Tyrosine).

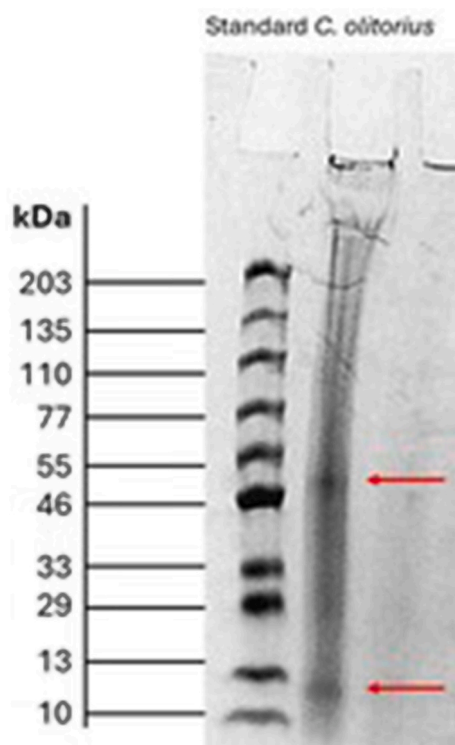


Fig. 1. Molecular weight distribution of proteins from Tunisian *C. olitorius* powdered leaves, obtained after NaOH extraction.

Cysteine, derived from Met, depends on Met availability. Therefore, Tyrosine and Cysteine will be considered “Indispensable amino acids” (IAA).

Amino acids values for *C. olitorius* are above the IAA requirements for adults (Table 6), established by the WHO/FAO/UNU (2007) report. Based on its balanced amino acid composition, *C. olitorius* shows potential as a new ingredient for the food industry and human consumption.

C. olitorius shows promise as an alternative protein source, despite conventional sources like eggs, beef, and soy providing the most IAAs.

Moringa leaves (*Moringa oleifera*) exceeds the IAAs content present in *C. olitorius*. However, *C. olitorius* has more IAAs than chickpeas (*Cicer arietinum* L.), per 100 g of dry matter (Table 7), despite chickpeas having slightly higher protein content (17–22 g/100 g). According to Boukid (2021), chickpeas are the third most cultivated pulse crop globally, with 15 million tons produced annually. Given its IAAs content, *C. olitorius* emerges as a promising candidate to provide plant-based proteins. It is important to note that chickpeas and *C. olitorius* belong to different food categories, affecting consumption volumes.

Furthermore, Sha'a et al. (2019) showed that concentrations of antinutrients in *C. olitorius* were found to be within acceptable levels for human consumption, making it a promising protein source.

3.5. Elemental analysis

The elemental composition is slightly higher than the compositions previously described (Idirs et al., 2010), enhancing the fact that *C. olitorius* general composition might be dependent of the growing

Table 7Indispensable amino acid content (g/100 g of dry matter) of *C. olitorius* leaves, and different conventional protein sources (FAO, 1970).

Indispensable amino acids	Egg (whole)	Beef (flesh)	Glycine max (seed)	Cicer arietinum (seed)	Corchorus olitorius (leaves)	Moringa oleifera (leaves)
SAA	2.76	1.81	1.17	0.50	0.71	1.20
AAA	4.71	3.63	3.65	0.20	1.89	1.85
Tryptophan	–	–	0.58	–	0.39	–
Isoleucine	2.99	2.18	2.05	1.00	0.90	1.48
Leucine	4.20	3.68	3.51	1.69	1.72	2.64
Lysine	3.32	4.03	2.88	1.55	1.15	1.82
Threonine	2.44	2.08	1.74	0.85	0.89	1.41
Valine	3.26	2.27	2.17	1.03	1.18	1.88
Histidine	1.16	1.55	1.14	0.60	0.49	0.69
ΣIAAs	24.83	21.23	18.90	7.41	9.31	12.96

SAA: sulphur amino acids (Methionine + Cysteine), AAA: aromatic amino acids (Phenylalanine + Tyrosine); IAAs: Indispensable amino acids. Results are reported on a dry matter basis, adjusted for the initial moisture content of samples.

Table 8Elements and oligo-elements content (mg/100 g of dry matter) from powdered Tunisian *C. olitorius* leaves (n = 3) and other leafy vegetables (Ayaz et al., 2006; Barminas et al., 1998).

Elements	Corchorus olitorius	Moringa oleifera	Brassica oleracea
Phosphorus (P)	312.3 ± 4.2	897	573
Calcium (Ca)	1342.3 ± 13.1	2041	1970
Magnesium (Mg)	410.3 ± 21.4	451	240
Potassium (K)	2348.0 ± 178.5	–	1350
Iron (Fe)	44.9 ± 2.4	29	7
Manganese (Mn)	8.7 ± 0.3	8	5
Copper (Cu)	1.1 ± 0.1	1	1
Zinc (Zn)	2.8 ± 0.1	26	4
Bore (B)	2.9 ± 0.1	–	–

location and conditions (Table 8).

Considering the high content of calcium (1342.3 ± 13.1 mg/100 g of dry matter), pectin present in *C. olitorius* may interact with calcium via the “egg-box” model upon contact with water (Cao et al., 2020). The calcium might bind with pectin’s free carboxyl groups, facilitating interactions upon hydration. This could enhance mobility, promote cross-linking, and partially explain the texturing effect of its leaves.

C. olitorius is also rich in potassium (2348.0 ± 178.5 mg/100 g of dry matter), which has benefits to the heart, kidney and other tissues (Weaver, 2013).

Comparisons of minerals content (Table 8) between *C. olitorius* leaves and some “superfood” such as Moringa (*Moringa oleifera*) and Kale (*Brassica oleracea*) shows the nutritional interest of this plant (Ayaz et al., 2006; Barminas et al., 1998). *C. olitorius* stands out for its high iron and potassium contents, which are the highest among the three plants compared. It also offers significant amounts of calcium and magnesium, though slightly less than *Moringa oleifera*. Although its phosphorus, zinc, and copper content are lower, the overall mineral composition makes it valuable for nutrient intake.

3.6. Chlorophyll and carotene

C. olitorius is relatively rich in Chlorophyll *a* and *b*, rivalling with its content in *Brassica juncea* leaves (Table 9).

Chlorophyll compounds prove to have several bioactive properties and health benefits such as antioxidant activity, anti-inflammatory,

Table 9Chlorophyll *a* and chlorophyll *b* content (mg/g of dry matter) of powdered Tunisian *C. olitorius* leaves (n = 3) and other leafy vegetables (Duma et al., 2014, pp. 262–265).

	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>
<i>Corchorus olitorius</i>	0.58 ± 0.03	0.19 ± 0.01
<i>Lactuca sativa</i>	0.14 ± 0.01	0.04 ± 0.02
<i>Spinacia oleracea</i>	1.04 ± 0.07	0.46 ± 0.04
<i>Brassica juncea</i>	0.69 ± 0.11	0.16 ± 0.03

antimutagenic activities and anti-obesity effects (Martins et al., 2023).

Carotene is a terpene contributing to photosynthesis, but it is also a precursor for vitamin A. It can act as antioxidant, but also supports vision, skin health, and protects cells from damage (Rao & Rao, 2007). The total carotene content of *C. olitorius* is 27.34 ± 0.90 mg/100 g of dry matter. Previously, Oboh et al. (2009), found the total carotenoid content of *C. olitorius* leaves to be 42.5 mg/100 g of dry matter, which is in line with the results of this study, even if slightly higher.

3.7. Antioxidant activity

Solvent has a significant impact on the antioxidant activities of *C. olitorius* extracts (Fig. 2). The methanolic and ethanolic extracts of *C. olitorius* present higher radical scavenging activity than the other extracts, with respectively IC₅₀ values of 111.46 ± 10.57 and 237.17 ± 13.73 µg/mL.

The described IC₅₀ values for DPPH radical scavenging activity of *C. olitorius* methanolic extracts range from 206.42 to 303.72 µg/mL (Biswas et al., 2020), while according to Ben Yakoub et al. (2018), extraction of *C. olitorius* with ethanol showed a IC₅₀ value of 48.66 ± 1.14 µg/mL. The current study found an IC₅₀ of 111.46 ± 10.57 µg/mL for methanolic extracts, comparable to ascorbic acid IC₅₀ value, ranging from 5 to 110 µg/mL (Mishra et al., 2012). These results confirm that *C. olitorius* leaves are an antioxidant source.

3.8. Secondary metabolites

As hypothesized after its proximate analysis, *C. olitorius* is possibly rich in secondary metabolites. LC-MS analysis allows the characterization of these metabolites.

Thirteen compounds were identified in the extract, visible on the chromatogram (Fig. 3) and listed in Table 10. The LC-MS analysis revealed various metabolic groups, including hydroxycinnamic acids like neochlorogenic and chlorogenic acids, known for their anti-mutagenic, and antioxidant activities (Sato et al., 2011). Among the detected compounds, sucrose was the most abundant. Its high concentration may result from the degradation of polysaccharides (PS), as suggested by the presence of glucose units.

Flavonoids represent a major group in this methanolic extract of *Corchorus olitorius*, with hyperoside (a quercetin-3-O-galactoside) being particularly prominent. In addition, several kaempferol derivatives (Kaempferol-7-O-glucoside and Kaempferol-3-O-glucoside) were detected in significant amounts, contributing to the bioactivity profile of the extract. These flavonoids are documented for their antioxidant, anti-cancer, and antiparasitic properties (B. Yang, Wen, et al., 2018). Linoleic acid, crucial for membrane structure and lipid metabolism, was also detected.

Triterpene saponins, such as echinocystic acid-3-O-glucoside, exhibit anti-inflammatory and apoptotic activities (Georgatza et al., 2016). Lastly, as a coumarin derivative, 4,6-Dimethyl-3(4'-hydroxyphenol)

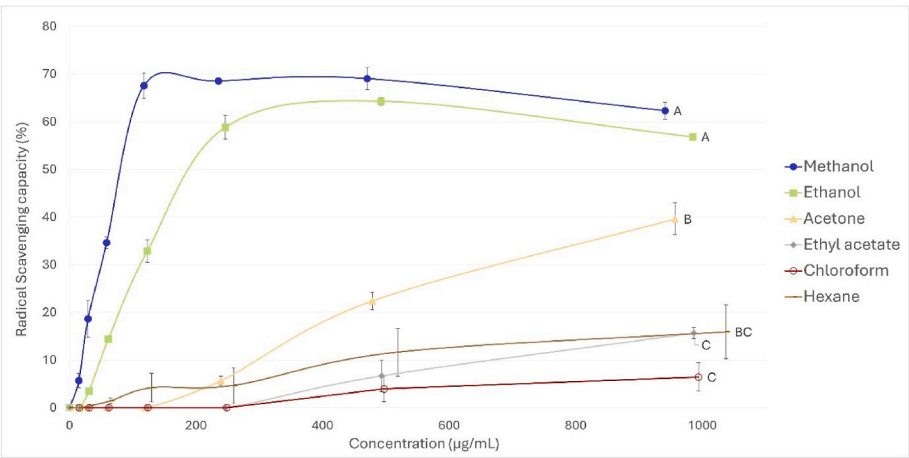


Fig. 2. DPPH radical scavenging activity (%) of *C. olitorius* extracts at different concentrations (µg/mL), obtained using various solvents. Data are presented as mean ± standard deviation (SD), n = 3. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test (p < 0.05).

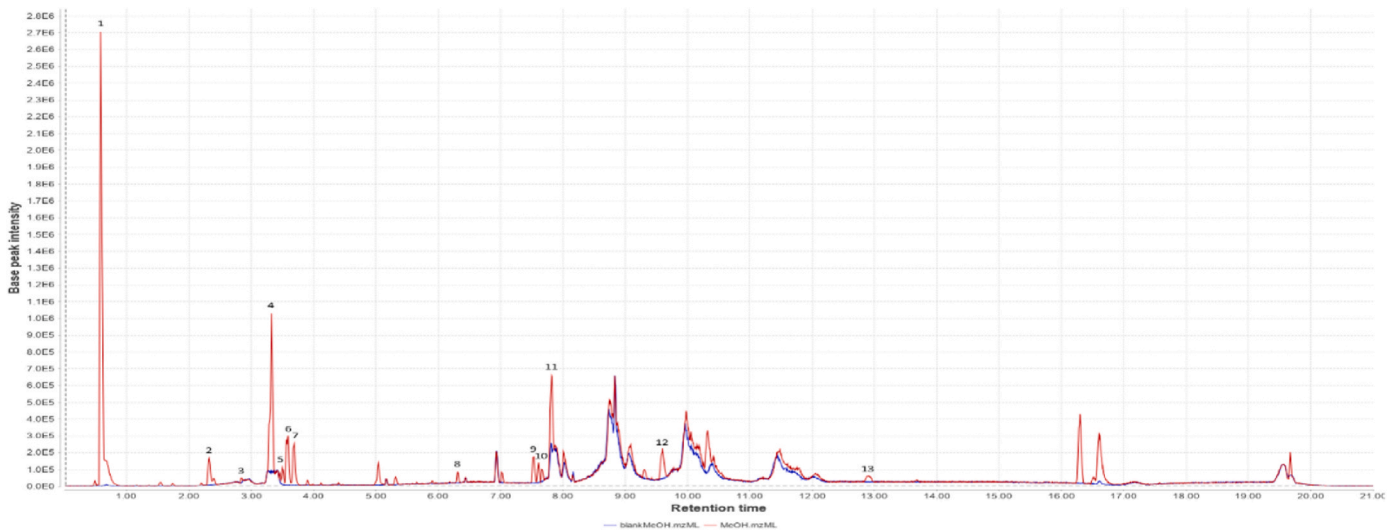


Fig. 3. LC/QTOF-MS chromatograms for methanol blank (blue) and *C. olitorius* methanolic extract (red), in negative electrospray ionization mode.

Table 10
Compounds identified by LC/QTOF-MS in negative electrospray ionization mode in methanolic extract of *Corchorus olitorius*.

Peak number	t _R (min)	Metabolite Class	Metabolite name	Experimental m/z	Molecular Formula
1	0.57	Disaccharide	Sucrose	341.11	C ₁₂ H ₂₂ O ₁₁
2	2.32	Phenolic acid (Hydroxycinnamic acid derivative)	Neochlorogenic acid	353.09	C ₁₆ H ₁₈ O ₉
3	2.84	Flavonoid (Flavonol glycoside)	Kaempferol-3-O-alpha-L-rhamnoside	431.19	C ₂₁ H ₂₀ O ₁₀
4	3.33	Flavonoid (Flavonol glycoside)	Quercetin-3-O-galactoside (Hyperoside)	463.09	C ₂₁ H ₂₀ O ₁₂
5	3.43	Flavonoid (Flavonol glycoside)	Jaceidin-7-rhamnoside	505.12	C ₂₄ H ₂₆ O ₁₂
6	3.50	Flavonoid (Flavonol glycoside)	Kaempferol-7-O-glucoside	447.09	C ₂₁ H ₂₀ O ₁₁
7	3.59	Flavonoid (Flavonol glycoside)	Kaempferol-3-O-glucoside (Astragalin)	447.11	C ₂₁ H ₂₀ O ₁₁
8	6.31	Triterpene saponin	Echinocystic acid-3-O-glucoside	635.34	C ₃₅ H ₅₈ O ₉
9	7.62	Linoleic acid and derivatives	–	293.21	C ₁₈ H ₃₀ O ₃
10	7.66	Flavonoid (Flavonol glycoside)	Apigenin-7-neohesperidoside (Rhoifolin)	577.27	C ₂₇ H ₃₀ O ₁₄
11	7.80	Coumarin (Benzopyrone derivative)	4,6-Dimethyl-3(4'-hydroxyphenol)coumarin	265.15	C ₁₇ H ₁₄ O ₃
12	9.60	Phenolic acid (Hydroxycinnamic acid derivative)	Chlorogenic acid	353.09	C ₁₆ H ₁₈ O ₉
13	12.90	Terpene glycoside	Pentose-Hexose + C ₅ H ₉	381.22	C ₁₆ H ₂₈ O ₁₀

t_R: Retention time.

coumarin might be linked to interesting biological activities including antimicrobial, anticancer, anti-inflammatory and antioxidant activities (Borges et al., 2005). While flavonoids are the primary secondary metabolites in the methanolic extract of *C. olitorius*, the analysis highlights a rich diversity of compounds with various biological activities.

As stated previously by Atalar et al. (2023), methanolic extracts of *C. olitorius* leaves demonstrates the presence of chlorogenic acid, hyperoside, quercetin-3-glucoside, and *trans*-ferulic acid. Another study showed that crypto-chlorogenic acid was the most abundant phenolic acid in *C. olitorius* leaves, and quercetin derivatives were the most abundant compounds within the flavonoids (Ncube et al., 2022). Finally,

5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, ascorbic acid, and α -tocopherol were also described in *C. olitorius* leaves (Azuma et al., 1999).

These findings support the hypothesis that the major metabolites in *C. olitorius* leaves are phenolic compounds, including chlorogenic acid and its derivatives, and flavonoids, particularly quercetin and kaempferol derivatives. These compounds significantly contribute to the numerous reported biological activities of this plant and help to confirm its health-promoting effects.

3.9. Limitations

This study has several limitations that should be considered. Although Sha'a et al. (2019) presented that concentrations of anti-nutrients in *C. olitorius* were found to be within acceptable levels, anti-nutritional compounds such as phytates, oxalates, and tannins were not assessed in the present study, and may impact the bioavailability of key micronutrients (Natesh et al., 2017). While the compositional data are comprehensive, amino acids bioavailability and digestibility were not evaluated, limiting the nutritional interpretation. All plant material was collected from a single site in Tunisia, without consideration of geographic or seasonal variability, which may influence composition.

Several studies have shown that the chemical composition of plants is strongly influenced by environmental factors such as soil type, climate, sunlight exposure, and water availability. These factors affect not only secondary metabolites (phenolic compounds or flavonoids) but also primary metabolites, such as polysaccharides, proteins, and lipids. Climatic and geographical variations have been reported to influence the accumulation of secondary metabolites (L. Yang, Wen, et al., 2018), and water stress has been shown to modulate phenolic biosynthesis (Chaves et al., 2003).

Primary metabolism is also affected as water and salinity stress have been reported to alter carbohydrate metabolism and reduce starch accumulation while increasing the production of soluble sugars that act as osmoprotectants (Seki et al., 2007). Finally, lipid profiles, especially membrane lipids, are known to shift under environmental stress (Upchurch, 2008). Such variations may impact the functional, nutritional, and biological properties of plant materials.

These findings emphasize the importance of considering environmental conditions when interpreting phytochemical and nutritional data. Future studies investigating the effects of abiotic stress on *Corchorus olitorius*' primary and secondary metabolites composition will be essential to better understand its biochemical variability and to optimize its use.

4. Conclusion

Corchorus olitorius showcases an impressive composition, rich in polysaccharides, proteins, and bioactive components, making it a promising option for multiple applications. With its nutrient dense profile, *C. olitorius* aligns with the growing demand for sustainable and health-oriented ingredients across sectors such as food, nutraceuticals, cosmetics, and pharmaceuticals.

This study provides one of the first detailed characterizations of *C. olitorius* leaves grown in Tunisia, emphasizing their high nutritional value. Notably, the amino acid profile exceeds WHO recommendations for essential amino acids, and the favourable ω -6/ ω -3 fatty acid ratio supports its relevance as a promising resource.

In the Tunisian context, *C. olitorius* holds particular promise as a locally adapted, nutrient-rich crop that can contribute to food security by diversifying diets and providing essential nutrients. Its cultivation supports sustainable agriculture by promoting the use of resilient plants, which is critical in regions facing climatic and resource challenges.

These findings support its potential use in the development of functional foods, nutraceutical supplements, and potentially bio-stimulants for agricultural applications.

Future research should focus on optimizing processing methods to enhance the extraction and utilization of its valuable components, such as polysaccharides, proteins and micronutrients. In particular, studies should investigate scalable and green extraction techniques, assess the bioavailability and functional efficacy of the extracted compounds, and develop effective formulations for *C. olitorius* based products. These efforts are essential to allow its application across the food, health, and agricultural sectors.

CRedit authorship contribution statement

Emma Bonnot: Writing – original draft, Visualization, Investigation, Data curation. **Ombéline Claux:** Writing – review & editing, Validation, Project administration. **Mounir Boulkout:** Funding acquisition, Conceptualization. **Agnès Rolland-Sabaté:** Formal analysis. **Maryline Abert-Vian:** Writing – review & editing, Supervision, Resources, Project administration, Methodology.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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