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**Phytochemical Characterization and Antioxidant Potential
of the Hydroethanolic Extract of *Cornulaca monacantha*
Delile: Prospects for Its Valorization as a Botanical
Ingredient for Liver-Function Support.**

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Dedication

To my beloved parents, especially my mother, thank you for everything, for your continuous support and encouragement throughout my journey. Mom, without you I could never have written this today. I love you so much; this success is yours.

I dedicate this thesis to my beloved family and my siblings, Ben Salem, Mohamed El Mehdi, Hamza, Mouna, Mounir, and Akram. Thank you for believing in me and for all the support you have given me.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning	Page(s) in thesis	PDF page(s)
AAE	Ascorbic Acid Equivalents	31, 33–34, 49–51, 61–62, 70, 73, 85	41, 43–44, 59–61, 71–72, 80, 83, 96
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)	2–3, 16, 25–27, 29–31, 33–35, 43, 52–56, 60–70, 72–74, 79–82, 85–86	9–10, 25, 34–36, 38–39, 41, 43–45, 53, 62–66, 70–80, 82–84, 89–92, 96–97
ALT	Alanine Aminotransferase	75–76, 83	85–86, 93
ANOVA	Analysis of Variance	76	86
AST	Aspartate Aminotransferase	75–76, 83	85–86, 93
CAT	Catalase	14, 75, 83	22, 85, 93
CC₅₀	Concentration causing 50% cytotoxicity	74–76, 80–81, 83	84–86, 90–91, 93
CCl₄	Carbon Tetrachloride	76	86
CUPRAC	Cupric Ion Reducing Antioxidant Capacity	66	76
CV	Coefficient of Variation	33–34, 41, 45–47, 49, 51, 53, 57, 65	43–44, 51, 55–57, 59, 61, 63, 67, 75
DPPH	2,2-Diphenyl-1-picrylhydrazyl	2–3, 16, 25–26, 29–31, 33–35, 43, 52, 57–74, 80–82, 84–86	9–10, 25, 34–35, 38–39, 41, 43–45, 53, 62, 67–84, 90–92, 94, 96–97
EC₅₀	Concentration producing 50% of the maximal effect	75–76, 83	85–86, 93
EMA	European Medicines Agency	1, 16–17, 20–21, 78, 80	8, 25–26, 29–30, 88, 90
FCR	Flavonoid Contribution Ratio	46, 48, 62, 67	56, 58, 72, 77
FDA	United States Food and Drug Administration	1, 78, 80	8, 88, 90
GAE	Gallic Acid Equivalents	24, 31, 33–34, 39–42, 46, 61–62, 68–69, 73, 85	33, 41, 43–44, 49–52, 56, 71–72, 78–79, 83, 96
GPx	Glutathione Peroxidase	14, 75, 83	22, 85, 93
GR	Glutathione Reductase	75	85
GSH	Reduced Glutathione	14, 75–76, 83	22, 85–86, 93
GSSG	Oxidized Glutathione	75, 83	85, 93
H₂O₂	Hydrogen Peroxide	75, 83	85, 93
HPLC	High-Performance Liquid Chromatography	28–29, 38, 43, 48, 72, 74–75, 80–81, 83	37–38, 48, 53, 58, 82, 84–85, 90–91, 93
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection	38, 43, 48, 72, 74–75, 80–81, 83	48, 53, 58, 82, 84–85, 90–91, 93

Abbreviation	Meaning	Page(s) in thesis	PDF page(s)
HO-1	Heme Oxygenase-1	14, 75	22, 85
HRMS	High-Resolution Mass Spectrometry	38, 48, 75, 80–81	48, 58, 85, 90–91
IC₅₀	Concentration required to inhibit 50% of the measured response	2–3, 14, 25, 30–31, 33–34, 53–63, 65, 67, 69–73, 80–81, 84–85	9–10, 22, 34, 39, 41, 43–44, 63–73, 75, 77, 79–83, 90–91, 94, 96
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use	29–31, 33, 44, 79, 81	38–39, 41, 43, 54, 89, 91
LDH	Lactate Dehydrogenase	75, 81, 83	85, 91, 93
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry	38, 43, 48, 56, 65–66, 72, 74–75, 80–81	48, 53, 58, 66, 75–76, 82, 84–85, 90–91
LOD	Limit of Detection	81–82	91–92
LOQ	Limit of Quantification	81–82	91–92
LPS	Lipopolysaccharide	14	22
MDA	Malondialdehyde	14, 75–76, 83	22, 85–86, 93
MIC	Minimum Inhibitory Concentration	14	22
MBC	Minimum Bactericidal Concentration	14	22
NF-κB	Nuclear Factor Kappa B	75	85
NMR	Nuclear Magnetic Resonance	38	48
NOAEL	No-Observed-Adverse-Effect Level	76, 83	86, 93
NQO1	NAD(P)H Quinone Dehydrogenase 1	14, 75	22, 85
NRF2	Nuclear Factor Erythroid 2–Related Factor 2	2, 75	9, 85
OECD	Organisation for Economic Co-operation and Development	76, 78, 80–81	86, 88, 90–91
PCI	Phenolic Contribution Index	62, 67	72, 77
QE	Quercetin Equivalents	25, 31–33, 44, 46–47, 61, 69–70	34, 41–43, 54, 56–57, 71, 79–80
R²	Coefficient of Determination	31–33, 39, 44, 49, 51, 61, 79–81, 85	41–43, 49, 54, 59, 61, 71, 89–91, 96
RE	Rutin Equivalents	31–34, 37, 44–47, 61–62, 69–70, 73, 85	41–44, 47, 54–57, 71–72, 79–80, 83, 96
ROS	Reactive Oxygen Species	73, 75, 83	83, 85, 93
SD	Standard Deviation	34, 41, 46, 51, 53, 57	44, 51, 56, 61, 63, 67
SOD	Superoxide Dismutase	14, 75, 83	22, 85, 93

Abbreviation	Meaning	Page(s) in thesis	PDF page(s)
TAC	Total Antioxidant Capacity	16, 28–29, 31–34, 43, 49–52, 61–65, 67, 70, 73–74, 79–82, 85	25, 37–38, 41–44, 53, 59–62, 71–75, 77, 80, 83–84, 89–92, 96
TBARS	Thiobarbituric Acid Reactive Substances	75	85
tert-BHP	Tert-Butyl Hydroperoxide	75, 83	85, 93
TFC	Total Flavonoid Content	16, 20, 29, 31–34, 37, 43–48, 52, 61–65, 67, 69–71, 73–74, 79–80, 82, 85	25, 29, 38, 41–44, 47, 53–58, 62, 71–75, 77, 79–81, 83–84, 89–90, 92, 96
TPC	Total Phenolic Content	16, 20, 29, 31–34, 37, 39–43, 46, 48, 52, 61–68, 70–74, 80, 82, 85	25, 29, 38, 41–44, 47, 49–53, 56, 58, 62, 71–78, 80–84, 90, 92, 96
UAE	Ultrasound-Assisted Extraction	42, 68	52, 78
UHPLC-HRMS	Ultra-High-Performance Liquid Chromatography–High-Resolution Mass Spectrometry	38	48
UPLC-HRMS	Ultra-Performance Liquid Chromatography–High-Resolution Mass Spectrometry	75, 81	85, 91
WHO	World Health Organization	16–18, 20–21, 78	25–27, 29–30, 88

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Introduction

INTRODUCTION AND PROBLEM STATEMENT

Dietary supplements and botanical preparations occupy an expanding interface between nutrition, preventive health, traditional medicine and natural product innovation. International frameworks, including those of the United States and the European Union, emphasize botanical identity, manufacturing consistency, chemical standardization, contaminant control, safety and the substantiation of physiological claims; presentation in a dosage form does not confer the evidentiary status of a medicine (United States Congress, 1994; European Parliament and Council, 2002; FDA, 2016; EMA/HMPC, 2022).

For an Algerian valorization objective, these international principles must be interpreted together with the national context. Any future botanical ingredient developed from an Algerian medicinal plant would require classification according to its composition, intended use and claims, as well as compliance with applicable national requirements governing food safety, consumer protection, public health, manufacturing quality and, where medicinal claims are made, pharmaceutical regulation. FDA and EMA guidance are therefore used as comparative quality benchmarks rather than substitutes for Algerian regulatory assessment.

Medicinal plants remain central to traditional healthcare systems and continue to provide bioactive scaffolds for drug discovery and botanical-product development. Nevertheless, ethnomedical use should be considered a scientifically valuable hypothesis-generating signal rather than proof of efficacy. Modern ethnopharmacology must combine traditional knowledge with reproducible botanical documentation, rational extraction, phytochemical profiling, mechanistic experimentation and toxicological assessment (Gurib-Fakim, 2006; Gaoue et al., 2021; Atanasov et al., 2021). This requirement is particularly important for plants used against complex disorders such as hepatic dysfunction, in which multiple metabolic, inflammatory and redox pathways interact.

The Sahara constitutes a major reservoir of stress-adapted flora. Its vegetation develops under extremely low and irregular rainfall, high evapotranspiration, intense solar radiation, wide thermal amplitudes, wind exposure and nutrient-poor, sandy, gypsum-rich or moderately saline soils. Despite low plant density, Saharan shrubs and subshrubs contribute to sand stabilization, habitat structuring and pastoral resources (Ozenda, 1991; Azizi et al., 2021; Bradai and Chenchouni, 2022). Environmental constraints may favour the accumulation of phenolic compounds, flavonoids, terpenoids, saponins and other protective metabolites involved in photoprotection, defence and redox homeostasis. However, phytochemical composition remains strongly influenced by genotype, geography, season, developmental stage, plant organ, drying and extraction conditions; desert origin alone cannot be equated with pharmacological superiority (Dai and Mumper, 2010; Khoddami et al., 2013; Azwanida, 2015).

Cornulaca monacantha Delile is a Saharo-Arabian xerophytic shrub currently assigned to Amaranthaceae, although it was historically classified within Chenopodiaceae. Described by Delile in the early nineteenth century, the species is distributed from North Africa and the Sahara through the Arabian Peninsula and Middle East to parts of south-western Asia. It is characterized by a highly branched woody habit, reduced glaucous leaves ending in a terminal spine and a strong adaptation to sandy and arid habitats (Delile, 1813; Maire, 1943; Boulos, 1999; Dobignard and Chatelain, 2011). In Algeria, it belongs to Saharan rather than steppe vegetation and occurs in sandy plains, dunes, wadi beds

and peripheral zones of gypsum-rich or moderately saline habitats. It is also browsed by camels and has pastoral significance.

Ethnomedicinal reports associate *C. monacantha* with jaundice, liver complaints, purgative uses and certain external applications. These reports justify scientific investigation but do not demonstrate hepatoprotective efficacy or oral safety. Traditional Algerian and Saharan plant preparations are commonly aqueous, particularly infusions, decoctions and water macerates. The 50:50 ethanolwater extract examined here was therefore not intended to reproduce a traditional preparation. It was selected as a reasoned exploratory laboratory system to broaden the recovery of polar and moderately polar constituents while retaining ethanol, a solvent more compatible with prospective food or botanical ingredient development than methanol or chlorinated solvents. Concentrated hydroethanolic extracts may nevertheless differ substantially from traditional preparations in composition and exposure (Boulos, 1983; Benhouhou, 2005; Ashour and Alsuwayt, 2019).

Previous studies have already investigated *C. monacantha* using aqueous, methanolic, ethanolic or optimized hydroethanolic approaches and have reported triterpenoid saponins, gallotannins, flavonoids, antioxidant isoflavones, phenylpropanoid amides and cornulacin (Kamel et al., 2000; Kandil and Grace, 2001; Mhiri et al., 2020; Badawy et al., 2024; Lemmadi et al., 2025; Eltamany et al., 2026). The novelty of the present work therefore does not reside in the first use of a mixed solvent, but in the transparent analytical evaluation of a defined 50:50 hydroethanolic macerate prepared from aerial material collected in Bennacer, southeastern Algeria, together with explicit unit correction, calibration appraisal and strict separation of chemical antioxidant evidence from biological claims.

The biological rationale for investigating this plant is also related to oxidative stress. Reactive oxygen and nitrogen species are normal components of cellular signalling, but excessive production or insufficient antioxidant defence can promote lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction and inflammatory amplification. The liver is particularly vulnerable because of its central functions in xenobiotic metabolism, nutrient processing and detoxification (Sies, 1985, 2015; Halliwell and Gutteridge, 2015). The NRF2-KEAP1 system is a major regulator of antioxidant and detoxification genes, yet activation of this pathway, restoration of glutathione or protection of hepatocytes cannot be inferred from chemical antioxidant assays alone (Itoh et al., 1997; Kensler et al., 2007; Cuadrado et al., 2019).

Antioxidant activity is multidimensional and no single test can provide a complete biological interpretation. The Folin-Ciocalteu assay yields a gallic-acid-equivalent reducing index rather than an absolute molecular concentration of phenolics. Aluminium chloride assays estimate selected flavonoid-reactive structures and are dependent on the calibration standard. The phosphomolybdenum assay measures global reducing capacity, whereas DPPH and ABTS evaluate reactivity toward chemically distinct radical systems. Results are influenced by solvent, pH, reaction time, radical concentration, steric accessibility and reaction kinetics (Folin and Denis, 1912; Blois, 1958; Singleton and Rossi, 1965; Brand-Williams et al., 1995; Re et al., 1999; Prieto et al., 1999; Huang et al., 2005; Prior et al., 2005; Apak et al., 2016; Gulcin, 2020). Consequently, complementary assays, correct standard-equivalent units and a strict distinction between interpolated and extrapolated IC₅₀ values are required.

Problem statement and research gap

Although the chemistry and biological potential of *C. monacantha* have been explored previously, the literature remains heterogeneous in plant origin, harvesting conditions, solvent composition, extraction intensity, calibration standards, assay conditions and biological models. Similar solvents have been used, notably ethanol-water systems optimized by ultra-sound assisted extraction, but these studies are not directly transferable to a conventional 24h 50:50 macerate from Bennacer. The specific gap addressed here is therefore methodological and contextual: the absence of a critically validated phytochemical antioxidant profile for this precise plant-material-solvent-method combination, accompanied by transparent treatment of calibration limitations and non-reached IC50 endpoints.

A second gap concerns interpretation. Positive phytochemical reactions and cell-free antioxidant results are frequently extended beyond their evidentiary scope. Molecular identification, cellular safety, hepatic models, in vivo toxicology, formulation stability and clinical assessment remain necessary before hepatoprotective, safety or product claims can be considered.

The central problem addressed in this work is whether the aerial-part 50:50 hydroethanolic macerate of *C. monacantha* collected in Bennacer displays a reproducible and analytically defensible phytochemical antioxidant profile that warrants further investigation, while maintaining a strict boundary between measured chemical responses and unproven biological effects.

Research question and objectives

Main research question: *Does this defined exploratory hydroethanolic extract display a phytochemical and antioxidant profile that can support subsequent analytical optimization and liver-directed biological validation?*

The general objective was to characterize the extract through qualitative phytochemical screening, total phenolic content, total flavonoid content, total antioxidant capacity, and complementary ABTS and DPPH assays. The study additionally aimed to evaluate calibration performance, correct standard equivalent units, assess analytical repeatability, compare the results descriptively with species specific literature and define the limitations separating cell-free antioxidant evidence from hepatoprotective validation.

The 50:50 ethanol-water proportion was treated as an exploratory choice rather than an optimized condition. Hemolytic, antihemolytic and nutritional analyses were initially planned but could not be performed because of limitations in facilities, analytical access, biological material, time and resources; they were therefore excluded from the experimental evidence and from the conclusions.

***Bibliographic Synthesis on
Cornulaca monacantha Delile***

I. Bibliographic Synthesis on *C. monacantha* Delile

I.1. General introduction

C. monacantha Delile is currently placed in Amaranthaceae, whereas classical floras and much of the older ethnobotanical literature treated it within Chenopodiaceae. This change reflects the modern inclusion of Chenopodiaceae within Amaranthaceae sensu lato rather than a change in the biological identity of the species. Nevertheless, the shift has practical consequences for literature interpretation: searches restricted to “Amaranthaceae” may miss older pharmacognostic, pastoral and ethnobotanical records indexed under “Chenopodiaceae”, while family-level generalizations drawn from older sources may rely on a historically broader and morphologically defined grouping.

Accordingly, older records remain relevant when the accepted species name, synonyms, locality and diagnostic morphology are concordant, but they should be reevaluated using current nomenclature. Modern databases, including POWO, GBIF and IPNI, recognize *C. monacantha* as the accepted name (Delile, 1813; POWO, 2026; GBIF, 2026; IPNI, 2026).

This species occupies a distinctive ecological position in the desert ecosystems of the Sahara, North Africa, the Arabian Peninsula, the Middle East, Iran, Afghanistan and western to south-western Pakistan. Plants of the World Online indicates that its native range extends from the Sahara to western and south-western Pakistan, where it mainly grows in desert and dry shrubland biomes (POWO, 2026).

From an ethnobotanical perspective, *C. monacantha* is known in several desert regions under Arabic vernacular names such as had, hâd, djouri, and, in some North African sources, under Tuareg names such as tahara. In Saharan and North African traditional medicine, the species is mainly cited for the management of jaundice, hepatic disorders, purgative uses, certain skin conditions such as scabies, and as a pastoral plant consumed by camels despite its spiny morphology (Benhouhou, 2005; Ashour and Alsuwayt, 2019).

In the context of the present research project, *C. monacantha* is of particular scientific interest for three main reasons. First, it belongs to a group of plants highly adapted to desert constraints, including drought, high temperature, poor soils, sandy substrates and, in some cases, gypsum-rich or moderately saline soils. Second, it has a traditional history of use related to hepatic disorders and jaundice. Third, several phytochemical investigations have reported the presence of triterpenoid saponins, flavonoids, gallotannins, polyphenols, phenolic acids, isoflavones and other bioactive compounds, which provide a scientific basis for investigating its antioxidant, cytoprotective and potentially hepatoprotective properties (Kamel et al., 2000; Kandil and Grace, 2001; Ashour and Alsuwayt, 2019; Badawy et al., 2024).

I.2. Systematic position and nomenclature

2.1. Current botanical classification

The currently accepted taxonomic classification of *C. monacantha* is as follows:

Taxonomic rank	Classification
Kingdom	Plantae
Phylum	Tracheophyta / Streptophyta, depending on the database
Class	Magnoliopsida / Equisetopsida, depending on the classification system
Order	Caryophyllales
Current family	Amaranthaceae
Former family	Chenopodiaceae
Genus	<i>Cornulaca</i> Delile
Species	<i>C. monacantha</i> Delile

GBIF classifies the species within Plantae, Tracheophyta, Magnoliopsida, Caryophyllales, Amaranthaceae, *Cornulaca*, *C. monacantha* (GBIF, 2026). Plants of the World Online also places the species in Amaranthaceae and confirms that the name is currently accepted (POWO, 2026).

The full scientific name is: ***C. monacantha* Delile**

The species was described in the botanical section of the **Description de l'Égypte, Histoire naturelle**, page 206, around 1813–1814 according to nomenclatural databases such as IPNI and GBIF (Delile, 1813; IPNI, 2026; GBIF, 2026).

I.2.2. Synonymy

Several heterotypic synonyms have been reported in modern taxonomic databases. Plants of the World Online notably lists the following synonyms:

- *Cornulaca amblyacantha* Bunge
- *Cornulaca arabica* Botsch.
- *Cornulaca monacantha* var. *diacantha* Maire
- *Kochia monacantha* Steud.
- *Salsola ferox* Delile ex Moq.

- *Traganum nudatum* Sieber ex Moq., reported as not validly published according to POWO

These synonyms are important because older or regional studies may refer to the plant under different names, especially in North African, Saharan, Arabian or Irano-Turanian floras (Maire, 1943; Boulos, 1999; Dobignard and Chatelain, 2011; POWO, 2026).

I.2.3. Meaning of the specific epithet

The specific epithet *monacantha* derives from botanical Greek or Latin and literally means “bearing a single spine”. This name refers to the presence of a single terminal spine on the leaves, which is one of the most characteristic morphological features of the species. The North African medicinal plant guide explicitly explains the term “*monacantha*” as “with one spine”, which is consistent with the spiny morphology of the plant (Benhouhou, 2005).

I.3. The genus *Cornulaca*

The genus *Cornulaca* Delile belongs to the family Amaranthaceae and includes plants adapted to arid, desert and semi-desert environments. Plants of the World Online recognizes several accepted species within the genus, including *C. monacantha*, *C. aucheri*, *C. ehrenbergii*, *C. korshinskyi*, *C. setifera* and other species depending on taxonomic treatment (POWO, 2026).

Species of the genus *Cornulaca* are generally low shrubs or subshrubs, often spiny, with reduced, sclerified or succulent leaves. They are adapted to water stress, intense solar radiation, wind exposure, nutrient-poor soils and, in some cases, salinity. These characters are typical of many Saharo-Arabian Amaranthaceae, formerly treated as Chenopodiaceae, which occur in open, sandy, stony or saline habitats.

In classical floras, the genus *Cornulaca* is often considered in relation to other xerophytic genera such as *Salsola*, *Traganum*, *Haloxylon*, *Anabasis*, *Atriplex* and *Zygophyllum*. These genera share several adaptive strategies to desert environments, including leaf reduction, spinescence, thick cuticles, glaucous surfaces, low growth form, developed root systems and resistance to grazing.

I.4. General botanical characteristics

I.4.1. Habit and plant architecture

C. monacantha is described as a desert shrub or subshrub, highly branched and woody, reaching approximately 50–60 cm in height. World Flora Online and Flora of Pakistan describe it as a woody, intricate shrub, green when fresh and becoming yellowish or whitish upon drying (WFO, 2026; Flora of Pakistan, 2001).

The Libyan botanical platform describes the species as a spreading, branched, woody shrub up to approximately 60 cm high, bearing short, thick, bluish-green leaves closely appressed to greyish, slender stems (Libyan Nature Platform, 2026).

This low and highly branched habit represents a typical adaptation to open Saharan environments. It reduces direct exposure to wind, limits water loss, traps sand particles, protects buds and increases resistance to grazing.

I.4.2. Stems

The stems are generally greyish, rigid, woody and highly branched, with a sometimes slender, filiform or tangled appearance. This architecture gives the plant the form of a compact small bush, sometimes cushion-like or sub-cushion-like, adapted to strong winds and sand movement.

The branches bear reduced and spiny leaves, giving the plant a defensive physiognomy. The woody tissues and peripheral parts may become yellowish or whitish after drying, a useful character for field identification during dry periods.

I.4.3. Leaves

The leaves of *C. monacantha* are short, thick, sessile, appressed or clasping at the base, and often bluish-green or glaucous. They are described as scale-like, fleshy or subfleshy, ending in **a single rigid terminal spine**. The Libyan Nature Platform reports that the leaves may reach approximately **10 × 2–2.5 mm**, being curved, narrowed from a clasping base towards a terminal spine, and bearing woolly axils (Libyan Nature Platform, 2026).

Leaf reduction and terminal spinescence reflect a dual strategy: water economy and defense against herbivores. Small leaves reduce the transpiring surface, while the spine limits excessive browsing. Despite this mechanical protection, the plant is consumed by camels, highlighting its pastoral value in several desert regions.

I.4.4. Indumentum and glaucous appearance

Botanical descriptions often mention that the plant is generally glabrous and glaucous, except at the leaf axils, which may be woolly. Glaucousness, often associated with epicuticular waxes, is a common adaptation among xerophytes. It may reduce excessive solar radiation absorption, limit water loss and protect tissues against wind abrasion and thermal stress.

I.4.5. Flowers

The flowers are generally solitary and located in woolly leaf axils. Some descriptions report orange-brown flowers occurring singly in the axils. The perianth is spiny or composed of coriaceous segments, with linear and hardened lobes. In desert Amaranthaceae/Chenopodiaceae, flowers are often small and inconspicuous, yet adapted to reproduction under conditions of low water availability.

I.4.6. Fruits and seeds

The fruit is generally an utricle or a fruiting structure enclosed within a persistent perianth. The seeds are reported as vertical, compressed or enclosed within a protective covering. These traits are common in Chenopodiaceae sensu lato. Seed dispersal may be influenced by wind, sand movement, local erosion and possibly animal passage.

I.4.7. Root system

General botanical sources describe *C. monacantha* as having a well-developed taproot. This type of root system is consistent with the ecology of desert shrubs, allowing access to deeper water reserves or more stable soil horizons. In perennial Saharan species, the root system is often the key determinant of survival during prolonged drought.

I.5. Biology, life cycle and phenology

C. monacantha is generally considered a **perennial** shrub or subshrub, although some works may describe it as annual or subannual depending on local conditions, phenological observations and degree of lignification. Modern botanical databases usually describe it as a **subshrub or shrub** (POWO, 2026).

Flowering is reported in some sources from late summer to autumn, particularly between August and November in regional floristic descriptions. However, in Saharan environments, phenology can vary considerably according to rainfall, soil moisture, temperature and grazing pressure. Perennial Saharan species often show opportunistic vegetative activity after rainfall, while retaining woody structures capable of surviving long unfavorable periods.

Functionally, *C. monacantha* may be classified among **desert chamaephytes** or subshrubs whose renewal buds remain close to the soil surface. In Saharan environments, chamaephytes and other perennial plants are major components of permanent vegetation, particularly where climatic conditions do not allow reliable annual regeneration. Studies conducted in sandy habitats of the northern Algerian Sahara show that these plant communities are species-poor but dominated by perennial, drought-active species adapted to extreme aridity and high rainfall variability (Azizi et al., 2021).

I.6. Ecological adaptations to desert environments

I.6.1. Adaptation to drought

C. monacantha displays several typical xerophytic features:

- low and branched growth form;
- reduced leaves;
- spiny leaves;
- glaucous surfaces;
- more or less succulent tissues;
- deep or robust root system;
- ability to survive in poor soils;
- reduction of transpiring surface area;
- resistance to wind and sand burial.

These adaptations allow the species to maintain minimal activity in habitats where rainfall is scarce, irregular and often insufficient to support dense vegetation.

I.6.2. Adaptation to wind and sand movement

In sandy and desert regions, wind is a major ecological factor. It causes tissue abrasion, seed movement, partial burial and sometimes root exposure. The low, branched and tangled habit of *C. monacantha* helps reduce mechanical damage and may promote local sand stabilization.

Some sources attribute to *C. monacantha* an ecological role in stabilizing sandy soils and limiting erosion. This role is particularly important in desert ecosystems, where perennial plants act as “fertility islands” by trapping fine particles, organic matter, seeds and moisture.

I.6.3. Tolerance to poor, sandy or gypsum-rich soils

The regions of Ouargla, Ghardaïa, Oued Righ and the northern Algerian Sahara are characterized by extreme aridity, often sandy, gypsum-rich, saline or alkaline soils, and high evapotranspiration. Studies on psammophytic communities of the northern Algerian Sahara indicate that these habitats include dunes, regs, wadi beds, sandy soils, saline soils and depressions, with generally species-poor but highly specialized flora (Azizi et al., 2021).

In the saline wetlands of Oued Righ, *C. monacantha* has been observed in the outer vegetation belts, on soils that are less saline and less humid than those colonized by strict halophytes. Koull and Chehma (2016) reported that *Zygophyllum album*, *Limoniastrum guyonianum*, *C. monacantha*, *Cistanche tinctoria*, *Mollugo nudicaulis* and *Sonchus maritimus* occur on less saline and less humid soils, forming the outermost belt of certain Saharan wetlands.

This suggests that *C. monacantha* should not necessarily be regarded as a strict halophyte, but rather as a Saharan xerophyte capable of tolerating several edaphic constraints, particularly sandy, poor, gypsum-rich or moderately saline soils.

I.7. Geographical distribution

I.7.1. Global distribution

Plants of the World Online reports a broad natural range for *C. monacantha*, extending from the **Sahara to western and south-western Pakistan**. The countries or regions explicitly cited as part of its native range include Afghanistan, Algeria, Chad, Egypt, the Gulf States, Iran, Iraq, Kuwait, Libya, Mali, Mauritania, Morocco, Niger, Oman, Pakistan, Palestine, Saudi Arabia, Sinai, Sudan/South Sudan, Tunisia, Western Sahara and Yemen (POWO, 2026).

This distribution corresponds to a **Saharo-Arabian to Saharo-Sindian chorological element**, meaning that the species is associated with arid environments extending from the African Sahara to the desert and subdesert regions of the Middle East and South-West Asia.

I.7.2. Distribution in Africa

In Africa, *C. monacantha* is present in several Saharan and North African arid countries, including Algeria, Egypt, Libya, Morocco, Tunisia, Mauritania, Mali, Niger, Chad, Western Sahara and Sudan/South Sudan according to POWO. This distribution makes it a representative species of North African desert formations (POWO, 2026).

The species is particularly important in Saharan landscapes, where it participates in open plant communities often associated with xerophytic species such as *Zygophyllum album*, *Salsola* spp., *Traganum* spp., *Stipagrostis* spp. and other plants adapted to sandy, stony or gypsum-rich soils.

I.7.3. Distribution in North Africa

In North Africa, *C. monacantha* is recorded in the floras of Algeria, Tunisia, Libya, Egypt, Morocco and Western Sahara. Classical and modern floristic references cited by POWO include, among others, Boulos for Egypt, Barry and Celles for Mauritania, Dobignard and Chatelain for North Africa, as well as several regional catalogues (Boulos, 1999; Barry and Celles, 1991; Dobignard and Chatelain, 2011; POWO, 2026).

In Libya, the Libyan Nature Platform reports the species from several regions, including the Zliten-Misurata plain, the Sirte plains, Jabal Nafusa, Marmarica, Al-Jufra, Fezzan and Gebel Al-Uweinat, confirming its affinity for different desert and subdesert landscapes.

I.7.4. Distribution in Algeria

In Algeria, *C. monacantha* is a Saharan species reported from the northern Sahara and various desert habitats. It is mentioned in studies concerning Ouargla, Ghardaïa, Oued Righ, Touggourt and, more generally, the spontaneous flora of the Algerian Sahara. International databases explicitly cite Algeria as part of the native range of the species (POWO, 2026).

In the north-eastern Algerian Sahara, phytoecological studies show that plant communities of sandy and desert soils are highly adapted to aridity, with habitats including hamadas, regs, sandy soils, saline soils, depressions and wadi beds. These habitats correspond to potential or observed habitats of *C. monacantha* in several regions of the northern Algerian Sahara (Azizi et al., 2021).

In the Oued Righ valley, Koull and Chehma (2016) reported *C. monacantha* among the species occurring in the outer vegetation belts of saline wetlands, on soils less saline and less humid than those dominated by strict halophytes. This observation is important because it places the species within an ecological transition between xerophytism, edaphic tolerance and peripheral vegetation of Saharan saline habitats.

I.8. Habitat ecology

I.8.1. Preferred habitats

C. monacantha is mainly found in:

- sandy areas;
- dunes and microdunes;
- sandy regs;
- wadi beds;
- sandy depressions;
- poor soils;
- open desert areas;
- margins of saline or gypsum-rich habitats;

- very arid Saharan or subdesert formations.

When referring to Ouargla or Oued Righ, it is preferable not to describe its Algerian habitat as “steppe”. These regions belong to the **northern Sahara**, with a hyperarid desert climate, rather than to the steppe in the strict bioclimatic sense.

I.8.2. Climate

The habitats of *C. monacantha* are characterized by:

- very low rainfall;
- high interannual variability;
- high evapotranspiration;
- very high summer temperatures;
- marked thermal amplitudes;
- frequent winds;
- prolonged drought;
- soils poor in organic matter.

In the northern Algerian Sahara, Azizi et al. (2021) described a hot desert climate of the BWh type according to the Köppen classification, with low rainfall, evapotranspiration far exceeding precipitation, and a dry period extending over almost the entire year.

I.8.3. Ecological role

C. monacantha probably plays several ecological roles in Saharan ecosystems:

1. **Stabilization of sandy soils** through its root system and low growth habit.
2. **Partial fixation of sand** around plant tussocks.
3. **Creation of microhabitats** for seeds, insects and small organisms.
4. **Contribution to camel grazing resources.**
5. **Maintenance of perennial biomass** in poor habitats.
6. **Ecological indicator** of certain desert or subdesert habitats.

In Saharan environments, perennial shrubs often play a structuring role that is disproportionate to their apparent biomass. They may concentrate resources, facilitate the establishment of other species and act as anchoring points in open desert landscapes.

I.9. Pastoral importance

C. monacantha is cited as a plant grazed by camels despite its spiny leaves. In several Saharan and Arabian regions, it constitutes a rangeland forage resource, particularly during periods when annual herbaceous plants are scarce.

The North African medicinal plant guide reports that the plant is used as a resource for camels and that, according to traditional knowledge, it may have a favorable effect on milk production in female camels (Benhouhou, 2005).

Ashour and Alsuwayt (2019) also reported that the aerial parts of *C. monacantha* are known in folk medicine for hepatic disorders and jaundice, as well as for purgative effects and a supposed ability to increase milk production in female camels.

This pastoral dimension is important for the present project because it indicates a long-standing traditional animal exposure to the plant. However, consumption by camels is not sufficient to demonstrate safety in humans. A plant may be well tolerated by one animal species and have different effects in humans, especially when administered as a concentrated extract.

I.10. Ethnobotanical and traditional medicinal uses

I.10.1. Uses against jaundice and hepatic disorders

The most relevant traditional use for the present study concerns jaundice and hepatic disorders. Several sources report the use of decoctions or preparations of aerial parts or leaves of *C. monacantha* for liver disorders or icterus. The North African medicinal plant guide mentions its use against icterus in Morocco and its digestive or purgative uses in North African traditional medicine (Benhouhou, 2005).

Ashour and Alsuwayt (2019) stated that the aerial parts of *C. monacantha* are used in desert and rural areas of North Africa and the Middle East for the traditional treatment of jaundice and other liver diseases.

However, it must be emphasized that an ethnobotanical report of hepatic use does not constitute clinical proof. It should rather be considered a starting hypothesis justifying phytochemical, pharmacological and toxicological investigations.

I.10.2. Purgative use

Purgative or laxative use has also been reported. This aspect is important because it may reflect the presence of saponins, irritating compounds or metabolites active on the gastrointestinal tract. For a future dietary supplement, this effect must be carefully monitored, as it may become undesirable at high doses or after prolonged use.

I.10.3. External use against scabies and skin disorders

Some North African sources mention external use against scabies or certain skin conditions. These uses may be related to antimicrobial, antiparasitic, anti-inflammatory or irritant activities. However, traditional topical application cannot be directly extrapolated to an oral formulation.

I.10.4. Veterinary and pastoral uses

The plant is cited in camel rangelands and is sometimes associated with increased milk production in female camels. This information is ethnoveterinary and pastoral. It could open complementary research perspectives concerning forage value, mineral composition or metabolic effects in camels, but it remains secondary to a human dietary supplement project.

I.11. Phytochemical data

I.11.1. Main classes of reported metabolites

Phytochemical studies on *C. monacantha* have reported several classes of compounds:

- triterpenoid saponins;
- flavonoids;
- isoflavones;
- gallotannins;
- polyphenols;
- tannins;
- phenolic acids;
- coumarins;
- alkaloids according to some preliminary screenings;
- phenylpropanoid amides;
- substituted tyramine derivatives.

Ashour and Alsuwayt (2019) summarized that the plant has already yielded 13 triterpenoid saponins, 6 flavonoid derivatives and 4 gallotannins. They also reported the presence of alkaloids, coumarins, triterpenoid saponins, flavonoids, polyphenols and tannins in preliminary phytochemical screenings.

I.11.2. Triterpenoid saponins

Triterpenoid saponins are among the major compound groups reported in *C. monacantha*. Kamel et al. (2000) isolated three new saponins together with known derivatives of oleanolic acid, hederagenin and 30methylphytolaccagenate. Their occurrence is pharmacologically relevant but also requires toxicological caution because saponins may exert surfactant, gastrointestinal, cytotoxic or hemolytic effects depending on structure, dose and route of exposure.

No validated human safe-intake threshold or species-specific no-observed-adverse-effect level has been established for crude *Cornulaca* preparations. Pastoral consumption by camels cannot be converted into a human safety threshold, and isolated saponin data cannot be directly extrapolated to a chemically complex extract. Consequently, any future oral product would require quantitative saponin profiling, in vitro membrane compatibility testing, acute and repeated dose studies and derivation of a preparation-specific safety margin. The present study did not perform hemolytic or oral safety experiments and therefore makes no safety claim.

I.11.3. Polyphenols and gallotannins

Kandil and Grace (2001) reported galloyltannin analogues from the aerial parts. Such compounds may contribute to reducing capacity and metal chelation, but their presence alone does not demonstrate bioavailability or protection in hepatic tissue.

I.11.4. Flavonoids and isoflavones

Flavonoids reported in *C. monacantha* include derivatives of quercetin, luteolin and other phenolic compounds. More recent work has also isolated a new isoflavone named cornulacin from the methanolic extract of *C. monacantha* collected in North Sinai, along with five known compounds: N-

trans-feruloyltyramine, N-trans-feruloyl-3'-methoxytyramine, N-trans-caffeoyl tyramine, Cannabisin F and lyciumamide D (Badawy et al., 2024).

Flavonoids are promising candidates in a “liver function support” approach because they may contribute to antioxidant activity, modulation of inflammation and cellular protection. However, their oral bioavailability is often limited and depends strongly on chemical form, plant matrix, formulation and intestinal microbiota.

I.11.5. Recent metabolomic data

A very recent study explored the metabolomic profile of a methanolic extract of *C. monacantha* in a model of LPS-induced neuroinflammation in Neuro-2a cells, with particular attention to the Nrf2 pathway (Eltamany et al., 2026). Although this study does not directly concern the liver, it is relevant because the Nrf2 pathway is also central to hepatic antioxidant defense.

For the present project, this finding suggests a possible mechanistic pathway. If *C. monacantha* activates or modulates the Nrf2 pathway in certain cellular models, it would be scientifically relevant to test this hypothesis in a hepatic model, for example HepG2, HepaRG or primary hepatocytes, by measuring Nrf2, HO-1, NQO1, GSH, SOD, CAT, GPx and MDA.

I.12. Reported biological activities

I.12.1. Antioxidant activity

Published antioxidant findings support the chemical reactivity of *C. monacantha* extracts and isolated constituents, but their rigor and comparability vary. Studies differ in solvent, concentration range, number and nature of replicates, reference antioxidant, radical preparation, incubation time and IC50 calculation. Some reports provide global spectrophotometric indices without complete uncertainty estimates, while isolated compound studies cannot be compared directly with crude extracts. The literature should therefore be considered supportive but not confirmatory evidence (Boussadia et al., 2024).

I.12.2. Hepatoprotective activity

Ashour and Alsuwayt (2019) reported screening of several fractions, including a HepG2based hepatic-injury component. The study suggests activity in an intermediate-polarity fraction, but interpretation is constrained by the available reporting of concentration ranges, replicate structure, metabolic competence of the model and the distinction between cytoprotection and direct radical scavenging. These data justify replication under fully documented dose-response conditions; they do not establish clinical hepatoprotection.

I.12.3. Antibacterial activity

Antibacterial studies are similarly method-dependent. Disc-diffusion results can be influenced by compound diffusion and should be supported by broth microdilution, MIC/MBC determination, adequate strain controls and replicated measurements (Boussadia et al., 2024). These findings remain secondary to the present antioxidant objective.

I.12.4. Anti-inflammatory activity

Reported anti-inflammatory claims require specification of the model, concentration, replication and endpoint, such as cyclooxygenase inhibition, nitric oxide production, cytokines or edema. Without such detail, mechanistic statements should remain hypotheses.

I.12.5. Antidiabetic and metabolic activity

Antidiabetic screening has been mentioned for *C. monacantha*, but its relevance to hepatic metabolism cannot be inferred without clearly described models, dose-response data, appropriate comparators and endpoints addressing glucose regulation, lipid metabolism, insulin sensitivity and hepatic injury. Across the biological literature, small or incompletely reported sample sizes, heterogeneous concentrations and different extract types limit direct synthesis. Prior claims are therefore treated as hypothesis-generating rather than as established efficacy.

I.13. Concluding synthesis

C. monacantha is a Saharo-Arabian species of botanical, ecological, pastoral and ethnomedicinal interest. Its reported saponins, gallotannins, flavonoids, isoflavones and related phenolics justify chemical and biological investigation. However, the existing evidence base is methodologically heterogeneous, and neither traditional use nor preliminary in vitro findings establishes a safe or effective liver-support ingredient. Progression toward human use requires extract-specific standardization, validated analytics, cellular safety, biologically relevant efficacy models and toxicological assessment.

Materials and Methods

II. Materials and Methods

II.1. General methodological approach

The present study experimentally evaluated the phytochemical profile and cell-free antioxidant properties of the aerial parts of *C. monacantha* Delile collected in southeastern Algeria. The completed workflow comprised plant collection and authentication, drying, grinding and storage, preparation of one hydroethanolic extract, qualitative phytochemical screening, determination of TPC, TFC and TAC, and DPPH and ABTS radical-scavenging assays. Hemolytic, antihemolytic and nutritional analyses were initially planned but were not performed; no human blood was collected and no nutritional result was generated. (WHO, 1998; WHO, 2003; WHO, 2011; EMA/HMPC, 2006; EMA/HMPC, 2022).

A 50:50 ethanol-water mixture was selected as a reasoned exploratory solvent system intended to recover a broad range of polar and moderately polar metabolites. Water promotes tissue swelling and extraction of highly polar constituents, whereas ethanol improves recovery of less-polar phenolic and related compounds. Ethanol was also preferred over methanol and chlorinated solvents because of its greater compatibility with prospective food and botanical-ingredient research. However, no preliminary solventratio comparison, solubility study or yield optimization was performed. (Harborne, 1998; Tiwari et al., 2011; Azwanida, 2015; Abubakar and Haque, 2020). The 50:50 ratio must therefore be regarded as an exploratory choice, not as an experimentally optimized condition and not as a direct reproduction of traditional Algerian aqueous preparations.

All completed assays used clean glassware, analytical-grade reagents, appropriate blanks or controls and the wavelength specified for each method. Results were expressed according to the standard actually used. The scope of interpretation was restricted to the measurements generated experimentally. (Singleton and Rossi, 1965; Bajorun et al., 1996; Prior et al., 2005; Huang et al., 2005; Gulcin, 2020 Sasidharan et al., 2011; EMA/HMPC, 2022).

II.2. Plant material

The plant material used in this study consisted of the aerial parts of *C. monacantha* Delile. The plant was collected from the region of Bennacer, located in south-eastern Algeria, during the period extending from September to December 2025. The aerial parts were manually harvested and included mainly leaves, young branches and associated above-ground structures. During collection, visibly damaged, highly degraded or externally contaminated plant fragments were avoided as far as possible. This selective collection approach is recommended for medicinal plant raw materials because damaged tissues may contain altered metabolites, microbial contaminants or degradation products that can influence the chemical and biological quality of the sample (WHO, 2003; WHO, 2011; EMA/HMPC, 2006).



Figure 1: Collection of the aerial parts of *C.monacantha*.

Botanical identification of the collected material was performed by Mr. Amar EDDOUD, University Kasdi Merbah Ouargla, Algeria, based on the diagnostic morphological characters of the species and by comparison with regional Saharan floras. This identification confirmed that the collected material belonged to *C. monacantha* Delile, family Amaranthaceae. Accurate botanical identification is essential in studies involving medicinal and nutraceutical plants, since the reproducibility, safety and biological interpretation of the results depend directly on the correct taxonomic identity of the plant material (WHO, 1998; WHO, 2003; Evans, 2009; Heinrich et al., 2018).

Immediately after harvesting, the fresh plant material was placed in clean paper bags and transported to the laboratory. Paper bags were preferred to plastic bags in order to allow aeration and reduce moisture accumulation, condensation and early microbial deterioration during transport. Good agricultural and collection practices recommend that collected medicinal plant materials should be protected from excessive humidity, contamination, fermentation and physical deterioration during transport from the field to the laboratory or processing site (WHO, 2003; EMA/HMPC, 2006; WHO, 2011).

Upon arrival at the laboratory, the plant material was rinsed with tap water to remove sand particles, dust, plant debris and other external impurities. A final rinse was then performed with distilled water in order to reduce residual mineral impurities and improve the cleanliness of the material before drying. Cleaning and removal of extraneous matter are important preparatory operations in the quality control of medicinal plant materials, particularly for desert plants exposed to dust, sand, mineral deposits and wind-borne contaminants (WHO, 1998; WHO, 2011; Kunle et al., 2012).

After washing, the plant material was drained and spread in a thin layer under laboratory conditions for air-drying. Drying was carried out at room temperature, away from direct sunlight, for approximately 7 days, until the material became sufficiently dry for grinding. Shade air-drying was adopted in order to reduce moisture content while limiting thermal degradation and photodegradation of sensitive phytochemicals, particularly phenolic compounds and flavonoids. Drying is a critical post-harvest step because residual moisture may promote microbial growth, enzymatic degradation and chemical instability of plant materials (Müller and Heindl, 2006; Rocha et al., 2011; WHO, 2011; Chua and Chou, 2003).



Figure 2: Drying of the *C.monacantha*

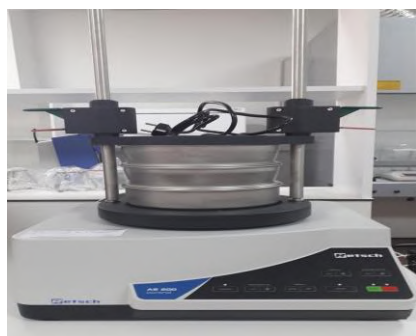
The dried aerial parts were ground using a refrigerated grinder to limit heat generation during comminution and reduce thermal alteration of sensitive constituents. Grinding was performed intermittently until a visually homogeneous powder was obtained, after which the powder was sieved to improve particle-size uniformity and solvent contact. The exact rotational speed, cycle duration and total grinding time were not instrumentally recorded. These missing technical parameters constitute a reproducibility limitation and should be documented in future work together with grinder model, sieve aperture and final particle-size distribution. (Harborne, 1998; Azwanida, 2015; Abubakar and Haque, 2020).



(1)



(2)



(3)

Figure 3: Grinding and sieving steps of *C.monacantha*.

The final powder was transferred into clean, dry and hermetically sealed glass containers. The containers were protected from light and stored at 4 °C until extraction and subsequent analyses. These storage conditions were adopted to limit moisture uptake, oxidation, contamination and photochemical degradation of bioactive compounds. Appropriate storage is a fundamental quality requirement for herbal

materials because temperature, light, oxygen and humidity can modify the chemical profile and biological activity of plant powders and extracts (WHO, 1998; WHO, 2011; EMA/HMPC, 2022).



Figure 4: Storage of *C.monacantha* powder.

II.3. Preparation of hydroethanolic extract

The hydroethanolic extract was prepared by macerating 10 g of dried, sieved aerial-part powder in 100 mL of ethanol-water (50:50, v/v), corresponding to a 1:10 solid-to-solvent ratio. The mixed solvent was selected to provide complementary extraction of polar and moderately polar constituents and to retain ethanol as a comparatively suitable solvent for prospective botanical-ingredient research. (Harborne, 1998; Handa et al., 2008; Tiwari et al., 2011; Azwanida, 2015).

This proportion was not derived from an optimization experiment. No comparison with other ethanol-water ratios, no preliminary solubility test and no response-surface or yield-optimization study was conducted. Accordingly, 50:50 represents a reasoned exploratory condition only. It should not be described as optimal, nor should the resulting concentrated extract be considered chemically equivalent to traditional Algerian infusions or decoctions. Future work should compare aqueous and graded hydroethanolic systems using extraction yield, marker compounds, TPC/TFC, antioxidant response, safety and scalability as joint selection criteria. (Dai and Mumper, 2010; Khoddami et al., 2013; Dent et al., 2013).

The hydroethanolic macerate was then recovered according to the laboratory procedure adopted for the study. The ethanolic fraction of the extract was removed under reduced pressure using a rotary evaporator at 40 °C. The use of reduced pressure and moderate temperature limits solvent boiling temperature and reduces the risk of thermal degradation of thermolabile compounds. Rotary evaporation is widely used in phytochemical laboratories for solvent removal after extraction, particularly when phenolic-rich or biologically active extracts are prepared (Handa et al., 2008; Abubakar and Haque, 2020).

After removal of ethanol, the remaining aqueous phase was freeze-dried using a lyophilizer. Lyophilization was used to remove water by sublimation and to obtain a dry extract with improved stability. Freeze-drying is commonly recommended for heat-sensitive biological and phytochemical matrices because it minimizes thermal stress and helps preserve structural and functional properties of labile compounds (Ratti, 2001; George and Datta, 2002; Shofian et al., 2011). The dry extract was

collected, weighed and stored at -20 °C until biological and analytical assays. Storage at -20 °C was selected to preserve the chemical integrity of the extract and reduce oxidative or enzymatic degradation before analysis (WHO, 2011; EMA/HMPC, 2022).

The extraction yield may be calculated according to the following equation:

$$\text{Extraction yield (\%)} = (\text{Weight of dry extract} / \text{Weight of dry plant powder}) \times 100$$

where the weight of dry plant powder corresponds to the initial mass used for extraction and the weight of dry extract corresponds to the final lyophilized extract obtained after solvent removal. Reporting extraction yield is important because it allows comparison between batches, extraction solvents and plant materials, and it is a basic parameter in phytochemical standardization (Handa et al., 2008; Azwanida, 2015; Abubakar and Haque, 2020).



(1)



(2)



(3)



(4)



(5)



(6)



(7)



(8)

Figure 5: Preparation of the hydroethanolic extract.

II.4. Qualitative phytochemical screening

Preliminary phytochemical screening was performed to detect the main classes of secondary metabolites present in the hydroethanolic extract of *C. monacantha*. The screening focused on flavonoids, terpenoids, polyphenols and saponins. These qualitative tests provide initial information on the phytochemical profile of the plant extract and guide the interpretation of subsequent quantitative and biological assays. Although qualitative phytochemical tests are not definitive structural analyses, they remain useful for preliminary classification of plant extracts and for selecting more specific chromatographic or spectrometric methods (Harborne, 1998; Trease and Evans, 2002; Sofowora, 2008; Tiwari et al., 2011).

II.4.1. Detection of flavonoids

The presence of flavonoids was investigated using the alkaline reagent test. Briefly, 2-3 mL of the plant extract were mixed with 2 mL of 10% sodium hydroxide solution. The development of an intense yellow coloration was considered indicative of flavonoids. This reaction is based on the formation of colored phenolate ions under alkaline conditions. The disappearance or reduction of the yellow color after acidification, when performed, further supports the presence of flavonoid compounds. Similar qualitative alkaline reagent tests have been reported in phytochemical screening protocols for medicinal plant extracts (Harborne, 1998; Trease and Evans, 2002; Sofowora, 2008; Tiwari et al., 2011).

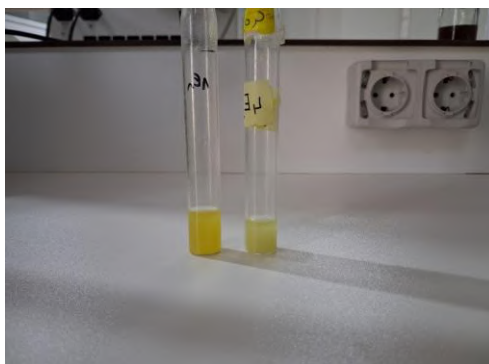


Figure 6: phytochemical screening test for Flavonoids.

II.4.2. Detection of terpenoids

The presence of terpenoids was assessed using the Salkowski-type reaction. Briefly, 0.5 g of the dry extract residue was mixed with 2 mL of chloroform. Then, 3 mL of concentrated sulfuric acid were carefully added along the wall of the tube to form a separate layer. The appearance of a reddish-brown ring at the interface between the two phases was interpreted as a positive indication of terpenoids. This test is commonly used as a qualitative reaction for terpenoid and steroidal constituents in preliminary phytochemical investigations (Harborne, 1998; Trease and Evans, 2002; Sofowora, 2008; Tiwari et al., 2011).



Figure 7: phytochemical screening test for terpenoids.

II.4.3. Detection of polyphenols

Polyphenols were detected using ferric chloride reagent. A few drops of 5% alcoholic ferric chloride solution were added to 2 mL of the plant extract. The appearance of a bluish-black, greenish-black or dark green coloration was considered indicative of phenolic compounds. This reaction is based on the complexation of ferric ions with phenolic hydroxyl groups, producing colored complexes. Ferric chloride screening is widely described as a classical qualitative test for phenols and tannins in plant extracts (Harborne, 1998; Trease and Evans, 2002; Sofowora, 2008; Tiwari et al., 2011).

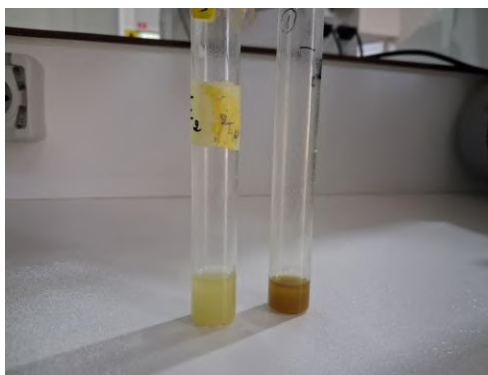


Figure 8: phytochemical screening test for polyphenols.

II.4.4. Detection of saponins

Saponins were detected using the frothing test. Briefly, 10 mL of the extract were placed in a test tube, vigorously shaken for 15 s and then allowed to stand for 15 min. The formation of a stable foam layer higher than 1 cm was considered indicative of saponins. The frothing property of saponins is related to their amphiphilic structure, which allows them to reduce surface tension and form persistent foam in aqueous media. This test is a classical qualitative screening method for saponin-containing plant materials (Trease and Evans, 2002; Sparg et al., 2004; Sofowora, 2008; Tiwari et al., 2011).



Figure 9: phytochemical screening test for saponins.

II.5. Determination of total phenolic content

The total phenolic content of the hydroethanolic extract was determined using the Folin-Ciocalteu colorimetric assay. This method is widely used for estimating the reducing capacity associated with phenolic compounds in plant extracts. The principle of the assay is based on the reduction of the phosphomolybdic-phosphotungstic reagent by phenolic compounds under alkaline conditions, resulting in the formation of a blue complex measurable spectrophotometrically (Singleton and Rossi, 1965; Slinkard and Singleton, 1977; Waterhouse, 2002; Prior *et al.*, 2005).

For the assay, 200 μL of diluted extract were mixed with 1000 μL of Folin-Ciocalteu reagent diluted 1:10 with distilled water. After vortexing, the mixture was incubated for 4 min at room temperature. Then, 800 μL of 7.5% sodium carbonate solution were added. The reaction mixture was incubated for 2 h at room temperature and protected from direct light. Absorbance was measured at 765 nm using a spectrophotometer. Similar reaction conditions and absorbance ranges have been used for plant extracts and food matrices rich in phenolic compounds (Boizot and Charpentier, 2006; Maisuthisakul *et al.*, 2007; Ainsworth and Gillespie, 2007).

Gallic acid was used as the reference standard. A calibration curve was prepared using gallic acid solutions at concentrations ranging from 0 to 200 $\mu\text{g/mL}$ under the same experimental conditions as the samples. Total phenolic content was calculated from the linear regression equation of the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract). Three analytical repetitions were performed for the extract. The expression of results as gallic acid equivalents is widely adopted in studies dealing with medicinal plants, foods, dietary supplements and antioxidant extracts (Waterhouse, 2002; Prior *et al.*, 2005; Ainsworth and Gillespie, 2007; Pérez *et al.*, 2023).

II.6. Determination of total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric method. This assay is based on the formation of stable complexes between aluminum ions and specific hydroxyl and keto groups of flavonoids. The resulting yellow complex can be measured spectrophotometrically and

used for the estimation of total flavonoid concentration (Bahorun et al., 1996; Zhishen et al., 1999; Chang et al., 2002; Djeridane et al., 2006).

Briefly, 1000 µL of diluted extract were mixed with 1000 µL of 2% aluminum chloride solution. The mixture was incubated for 10 min at room temperature and protected from light. Absorbance was then measured at 430 nm using a spectrophotometer. Quercetin was used as the reference standard. A calibration curve was prepared using quercetin solutions at concentrations ranging from 0 to 40 µg/mL under the same conditions as the extract. Total flavonoid content was calculated from the linear regression equation of the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract). Two analytical repetitions were performed. Quercetin is commonly used as a standard for total flavonoid assays because it forms stable complexes with aluminum chloride and is representative of flavonol-type structures (Bahorun et al., 1996; Chang et al., 2002; Djeridane et al., 2006).

II.7. Evaluation of antioxidant activity

The antioxidant activity of the hydroethanolic extract was evaluated using complementary *in vitro* assays, including DPPH radical scavenging activity, ABTS radical cation decolorization assay and phosphomolybdenum total antioxidant capacity assay. The use of several assays is recommended because antioxidant activity is a multidimensional property and no single chemical test can fully describe the antioxidant potential of a complex plant extract. Different assays are based on different reaction mechanisms, including hydrogen atom transfer, electron transfer, radical scavenging and reducing power (Huang et al., 2005; Prior et al., 2005; Apak et al., 2016; Gulcin, 2020).

II.7.1. DPPH radical scavenging assay

The DPPH radical scavenging activity was evaluated using a spectrophotometric method based on the reduction of the stable free radical DPPH. The DPPH radical has an intense purple color with maximum absorbance around 517 nm. In the presence of an antioxidant compound capable of donating hydrogen atoms or electrons, DPPH is reduced and the solution becomes less intensely colored (Blois, 1958; Brand-Williams et al., 1995; Molyneux, 2004; Kedare and Singh, 2011).

Briefly, 0.5 mL of sample solution was mixed with 3 mL of DPPH solution at a concentration of 0.06 mmol/L. The mixture was incubated for 30 min in the dark at room temperature. Absorbance was then measured at 517 nm. A blank and a control were prepared under the same conditions. Trolox or ascorbic acid may be used as reference antioxidants; however, they must not be considered identical standards. Trolox is a water-soluble analogue of vitamin E, whereas ascorbic acid is vitamin C. Results may therefore be expressed either as Trolox equivalents or as ascorbic acid equivalents, depending on the standard actually used for calibration (Re et al., 1999; Prior et al., 2005; Gulcin, 2020).

The percentage of DPPH radical inhibition may be calculated as follows:

$$\text{DPPH inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the DPPH solution without extract and A_{sample} is the absorbance of the DPPH solution in the presence of extract. When a dose-response curve is established, the IC₅₀ value may also be calculated. The IC₅₀ corresponds to the concentration of extract required to

inhibit 50% of the DPPH radical and is commonly used to compare radical scavenging potency between samples (Molyneux, 2004; Kedare and Singh, 2011; Gulcin, 2020; Gulcin, 2023).



The Sample



Ascorbic acid

Figure 10: Antioxidant activity assay using the DPPH method.

II.7.2. ABTS radical cation decolorization assay

The ABTS assay was used to evaluate the ability of the extract to scavenge the ABTS radical cation. The ABTS^{•+} radical has a blue-green color and absorbs strongly at 734 nm. Antioxidant compounds reduce ABTS^{•+}, leading to a decrease in absorbance proportional to the antioxidant capacity of the sample (Miller et al., 1993; Re et al., 1999; Arts et al., 2004; Zhen et al., 2016).

A stock ABTS^{•+} solution was prepared by mixing equal volumes of ABTS solution and potassium persulfate solution. In the present protocol, an 8 mM ABTS solution was mixed with a 3 mM potassium persulfate solution. The mixture was kept in the dark for 16 h to allow radical generation. Before use, the ABTS^{•+} solution was diluted with distilled water until an absorbance of approximately 0.70 ± 0.02 at 734 nm was obtained. For the assay, 10 μ L of sample solution were mixed with 990 μ L of the diluted ABTS^{•+} solution. The reaction mixture was vigorously mixed and incubated for 20 min in the dark at room temperature. Absorbance was measured at 734 nm. Trolox was used as the comparative standard when results were expressed as Trolox equivalents (Re et al., 1999; Prior et al., 2005; Zhen et al., 2016).

The percentage of ABTS radical inhibition may be calculated as follows:

$$\text{ABTS inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the ABTS^{•+} solution without extract and A_{sample} is the absorbance measured in the presence of extract.



ABTS radical



Ascorbic acid



The sample

Figure 11: Antioxidant activity assay using the ABTS method .

II.7.3. Phosphomolybdenum total antioxidant capacity assay

The total antioxidant capacity of the extract was determined using the phosphomolybdenum method. This assay is based on the reduction of molybdenum (VI) to molybdenum(V) by antioxidant compounds under acidic conditions, followed by the formation of a green phosphate/Mo(V) complex measurable spectrophotometrically (Prieto et al., 1999; Umamaheswari and Chatterjee, 2007; Prior et al., 2005; Gulcin, 2020).

Briefly, 0.1 mL of sample solution was mixed with 1 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes were capped and incubated in a water bath at 95 °C for 90 min. After incubation, the samples were cooled to room temperature and absorbance was measured at 695 nm. Ascorbic acid was used as reference standard. Results may be expressed as milligrams of ascorbic acid equivalents per gram of dry extract or as percentage antioxidant capacity according to the selected calculation approach (Prieto et al., 1999; Umamaheswari and Chatterjee, 2007; Prior et al., 2005; Gulcin, 2020).

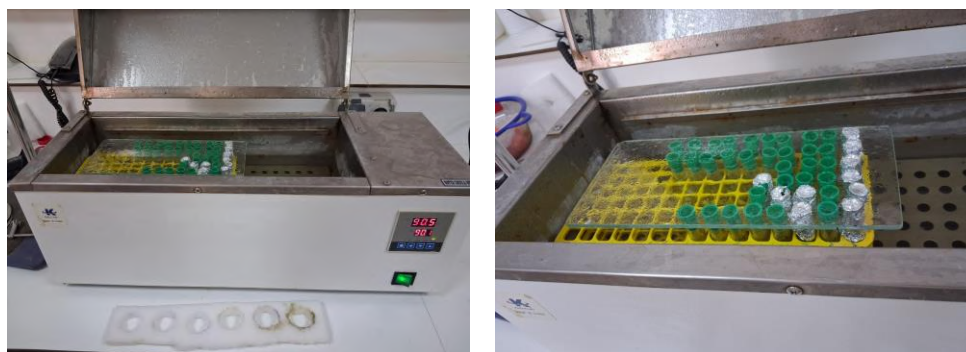


Figure 12: Antioxidant activity assay using the TAC method.

II.8. Planned hemolytic and antihemolytic assessments not performed

Hemolytic and antihemolytic assays were initially included in the research plan to examine erythrocyte membrane compatibility and protection against oxidant-induced hemolysis (Niki, 1990; Niki et al., 1998; Dai et al., 2006; Takebayashi et al., 2010). These experiments could not be conducted because of limitations in facilities, biological material, time and logistical resources.

No human blood was collected, no volunteer was enrolled and no erythrocytebased experiment was performed. Consequently, institutional ethics approval was not sought for a procedure that did not take place, and the present thesis reports no hemolysis, hemocompatibility or antihemolytic result.

The protocols previously considered for these assays are not presented as completed methods. Future implementation would require prior approval by a competent ethics committee, written informed consent, a registered protocol, anonymized sample handling, biosafety procedures and full reporting of participant eligibility and blood-processing conditions.

Interpretive consequence: the current extract cannot be described as hemocompatible or protective against oxidative hemolysis.

II.9. Planned nutritional analyses - not Performed

Determination of total lipids, nitrogen, crude protein and water-soluble vitamins by HPLC was initially planned to support evaluation of the aerial-part powder as a possible botanical ingredient. These analyses could not be completed because of limitations in equipment availability, analytical access, time and resources.

No gravimetric lipid value, Kjeldahl nitrogen or protein result, and no chromatographic vitamin concentration was obtained. The Folch, Kjeldahl and HPLC procedures considered during planning are therefore not reported as methods actually performed.

Interpretive consequence: the present study provides no evidence concerning nutritional composition and cannot support nutritional claims. Proximate composition, mineral content, vitamins, contaminants and antinutritional factors remain priorities for a future product-oriented study.

II.10. Methodological scope

The experimental evidence of this thesis is restricted to qualitative phytochemical screening, TPC, TFC, TAC, ABTS and DPPH measurements.

Consequences of incomplete planned work

The inability to perform the planned erythrocyte-based and nutritional experiments is reported as a study limitation rather than concealed or replaced by literature-derived values. This distinction preserves the integrity of the evidence chain:

- no ethics approval number is applicable because no human-derived material was collected or analyzed;
- no statement on hemolytic safety or antihemolytic protection is supported;
- no statement on lipid, protein or vitamin composition is supported;
- future studies must begin with prospectively approved and fully documented protocols.

These planned analyses remain relevant to botanical-ingredient development, but they belong to the future-validation program rather than to the completed experimental methods

Planned HPLC vitamin analysis not performed

Vitamin profiling by HPLC was not conducted. Consequently, no sample preparation, injection, calibration or quantitative vitamin result is claimed in this thesis. Future work should validate the chromatographic method for the plant matrix, assess recovery and stability of light-sensitive vitamins, and report concentrations on a clearly defined dry-mass basis.

The absence of these nutritional data limits immediate product formulation. Any future botanical ingredient would require a separate compositional dossier including proximate composition, minerals, vitamins, contaminants, pesticide residues, microbiological quality and relevant antinutritional factors.

II.11. Quality control and analytical reliability

For all spectrophotometric and chromatographic assays, reagent blanks and appropriate controls were used. Calibration curves were established using reference standards under the same analytical conditions as the samples. The linearity of each calibration curve was assessed using the regression coefficient. Results were expressed based on the corresponding standard curve and corrected for dilution factors when required. The use of calibration standards, blanks and quality controls is essential to ensure analytical reliability and comparability of results (ICH, 2005; ICH, 2023; Eurachem, 2014).

Where possible, measurements should be performed at least in duplicate or triplicate. The use of replicate measurements improves the reliability of analytical results and allows the calculation of mean values and dispersion parameters. For future validation of quantitative assays, analytical performance parameters such as linearity, precision, accuracy, limit of detection, limit of quantification and robustness

may be evaluated in accordance with internationally recognized validation guidelines (ICH, 2005; ICH, 2023; AOAC International, 2016).

II.12. Statistical analysis

All experimental results should be expressed as mean $\pm$ standard deviation when at least three independent measurements are available. For assays performed in duplicate, the two values may be reported individually or summarized as mean $\pm$ range, while clearly indicating the limited number of repetitions. Calibration curves should be constructed by linear regression, and the regression equation and coefficient of determination should be reported. Appropriate reporting of replicate structure, dispersion and calibration parameters is essential for transparent analytical interpretation (Miller and Miller, 2018; ICH, 2023).

When comparing several extract concentrations or experimental groups, normality and homogeneity of variance should be checked before selecting parametric or non-parametric tests. Dose-response data, including DPPH inhibition, ABTS inhibition, hemolysis and anti-hemolytic protection, may be analyzed using regression approaches. IC₅₀ or HT₅₀ values may be estimated from fitted dose-response or kinetic curves when the data structure allows reliable modeling (Motulsky and Christopoulos, 2004; Sebaugh, 2011; Ritz et al., 2015).

II.13. Methodological note

In the antioxidant assays, Trolox and ascorbic acid should be treated as two distinct reference standards. Trolox is a water-soluble analogue of vitamin E and is commonly used to express Trolox equivalent antioxidant capacity. Ascorbic acid is vitamin C and may be used independently as another antioxidant reference. Therefore, the expression “Trolox = ascorbic acid” should be avoided. Results should be expressed as Trolox equivalents only when Trolox is actually used for calibration, or as ascorbic acid equivalents when ascorbic acid is used (Re et al., 1999; Prior et al., 2005; Gulcin, 2020).

Similarly, for the hemolysis assay, the percentage of hemolysis should be calculated using both the negative control and the positive control. The recommended formula is:

$$\text{Hemolysis (\%)} = [(A_{\text{sample}} - A_{\text{negative control}}) / (A_{\text{positive control}} - A_{\text{negative control}})] \times 100$$

This equation allows normalization of the sample response between spontaneous hemolysis and complete hemolysis and is more appropriate than formulas that do not account for the absorbance of the negative control (Henkelman et al., 2009; Takebayashi et al., 2010).

Results and Discussion

III. Results and Discussion

III.1. Analytical Quality Control and Data Validation

Rationale and analytical validation logic: This subsection precedes the biological interpretation and defines the analytical reliability of the results obtained for the hydroethanolic extract of *C. monacantha*. In phytochemical and antioxidant investigations, numerical results are scientifically meaningful only when calibration behaviour, blank/control treatment, unit attribution, repeatability, and IC50 decision rules are explicitly verified. This requirement is particularly important for colorimetric plant-extract assays because the Folin-Ciocalteu, aluminium chloride/rutin, phosphomolybdenum, ABTS and DPPH procedures do not measure single molecular entities; rather, they generate standard-equivalent indices or radical-scavenging endpoints whose interpretation depends on the chemical standard, wavelength, reaction medium, and calculation model (Folin and Denis, 1912; Folin and Ciocalteu, 1927; Singleton and Rossi, 1965; Blois, 1958; Brand-Williams et al., 1995; Re et al., 1999; Prior et al., 2005; Apak et al., 2016). Accordingly, the present quality-control step was designed to document the calibration equations, correct the final units, recalculate ABTS and DPPH inhibitions from raw absorbance values, and distinguish interpolated IC50 values from extrapolated estimates.

Analytical principle: The conversion of absorbance into concentration-equivalent values was performed only through the corresponding standard curve. TPC was therefore expressed as gallic acid equivalents (GAE), TFC as rutin equivalents (RE), and TAC as ascorbic acid equivalents (AAE). This convention follows the metrological logic of colorimetric assays: the unit is defined by the calibrator, not by the broad class of compounds expected in the extract. The correction of TFC from mg QE/g extract to mg RE/g extract is therefore mandatory because the calibration curve was prepared using rutin rather than quercetin (Zhishen et al., 1999; Chang et al., 2002; Prior et al., 2005).

IC50 decision principle: For ABTS and DPPH, inhibition percentages were recalculated using the experimental control absorbance. The IC50 was treated as a true interpolated endpoint only when 50% inhibition was experimentally bracketed by two measured concentrations. When 50% inhibition was not achieved within the tested range, the result was reported as greater than the highest tested concentration, and any value obtained by extrapolation was retained only as an indicative estimate. This distinction prevents overinterpretation of weak dose-response behaviour and is consistent with current recommendations for antioxidant-capacity reporting and analytical-method validation (Prior et al., 2005; Sharma and Bhat, 2009; ICH, 2023a, 2023b).

Calibration curve quality control: The gallic-acid curve showed excellent linearity ($R^2 = 0.9995$). By contrast, the rutin and ascorbic-acid curves gave R^2 values of 0.9595 and 0.9696. These values are below the performance expected for defensible quantitative calibration. Because the original measurements could not be repeated, the associated TFC and TAC values were retained only as provisional exploratory estimates, not as fully validated quantitative results. They should not be used to define specifications, compare small differences among studies or support product claims. Re-optimization and repetition of both calibrations are required.

Table 1. Calibration curve quality control.

Assay	Standard	Wavelength (nm)	Equation	R2	Range	Blank/control treatment	Acceptance comment
TPC	Gallic acid	765	$y = 0.009444x - 0.0053$	0.9995	0-200 $\mu\text{g/mL}$	Blank included; near-zero absorbance	Excellent linearity
TFC	Rutin	430	$y = 0.0042x - 0.0138$	0.9595	0-40 $\mu\text{g/mL}$	Blank included; unit corrected to rutin equivalents	Moderate linearity; calibration should be improved
TAC	Ascorbic acid	695	$y = 0.001203x - 0.006476$	0.9696	0-100 $\mu\text{g/mL}$	Blank included; zero absorbance at 0 $\mu\text{g/mL}$	Acceptable for preliminary estimation; improvement recommended

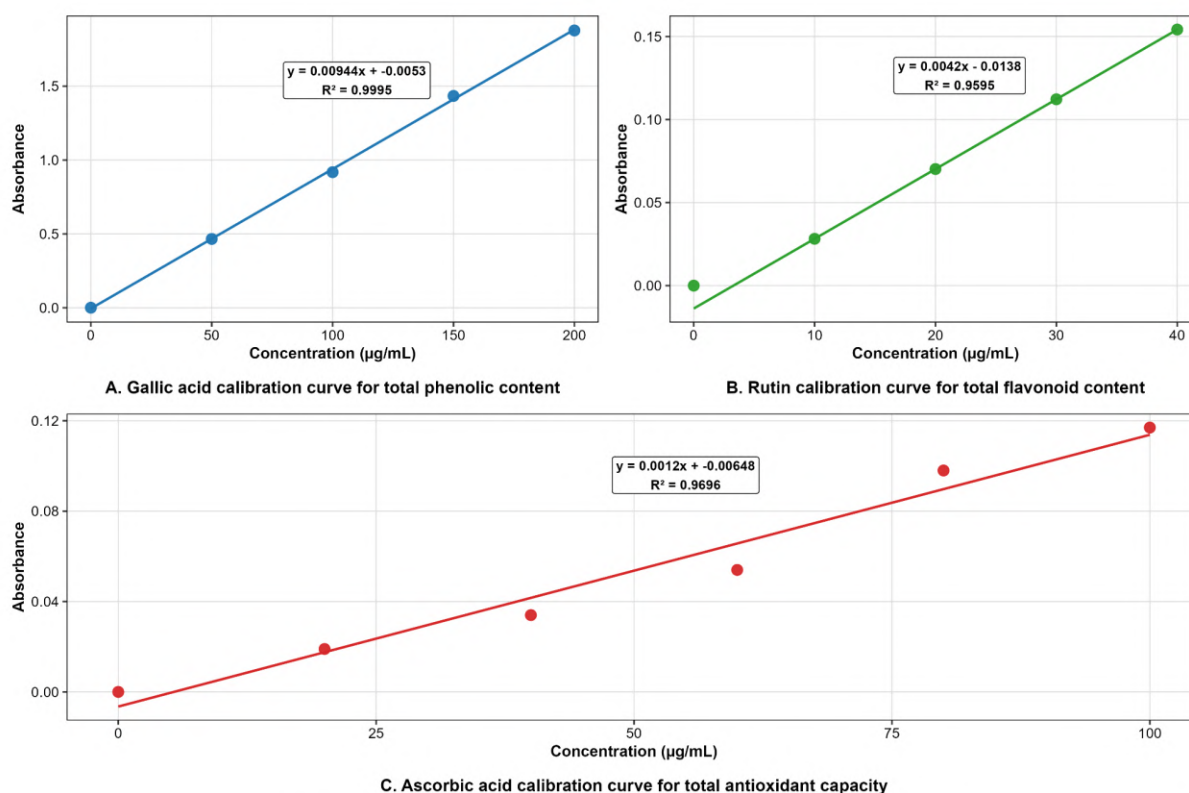
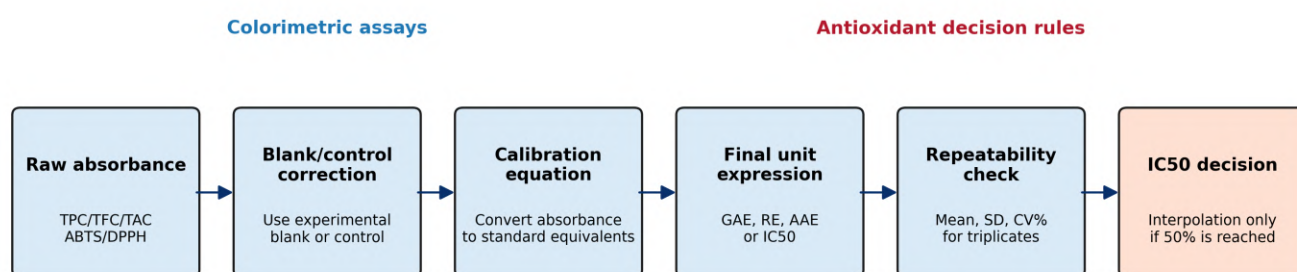


Figure 13. Calibration curves used for analytical quality control of *C. monacantha* extract.

Correction of units and expression of validated results: The corrected units and final expression of the analytical endpoints are presented in Table 2. The most important correction concerns the total flavonoid content: because rutin was the calibration standard, the result must be reported as mg RE/g extract. Reporting the same value as mg QE/g extract would be chemically and metrologically incorrect, because rutin and quercetin do not define identical calibration scales.

Table 2. Unit correction and expression of results.

Parameter	Abbreviation	Standard/reference	Correct unit	Correction made	Final result
Total phenolic content	TPC	Gallic acid	mg GAE/g extract	Calculated from gallic acid curve	21.79 +/- 0.50 mg GAE/g extract
Total flavonoid content	TFC	Rutin	mg RE/g extract	Corrected from QE to RE because rutin was used	8.42 +/- 0.27 mg RE/g extract
Total antioxidant capacity	TAC	Ascorbic acid	mg AAE/g extract	Calculated from ascorbic acid curve	17.43 +/- 0.42 mg AAE/g extract
ABTS radical scavenging	ABTS	Ascorbic acid	IC50 in µg/mL	Inhibition recalculated from raw absorbance	Extract: 185.93 µg/mL
DPPH radical scavenging	DPPH	Ascorbic acid	IC50 in µg/mL	Inhibition recalculated; IC50 decision corrected	Extract: IC50 > 1000 µg/mL; extrapolated ~1076.29 µg/mL



Quality-control message: units are assigned by the calibration standard; extrapolated IC50 values are not equivalent to true interpolated IC50 values.

Figure 14. Analytical workflow from raw absorbance to corrected concentrations, final units and IC50 reporting rules.

Interpretation of quantitative outputs. TPC is supported by an excellent calibration and may be interpreted as the most analytically robust quantitative index in the dataset. TFC and TAC retain the correct standard-equivalent units, but the low calibration R^2 values compromise quantitative precision. Their means and within-run CV values describe the available measurements only; good repeatability of sample readings does not compensate for inadequate calibration fit. (Thompson et al., 2002; Magnusson and Ornemark, 2014; ICH, 2023a).

The TFC value of 8.42 ± 0.27 mg RE/g and the TAC value of 17.43 ± 0.42 mg AAE/g are therefore reported as provisional screening estimates. A future validated analysis must repeat standards and samples, increase and appropriately distribute calibration levels, inspect residuals, test lack of fit, assess accuracy/recovery and establish precision and robustness.

DPPH decision: because 50% inhibition was not reached, the only valid extract result is $IC_{50} > 1000$ µg/mL. The extrapolated value must not enter derived efficiency indices.

Table 3. Analytical repeatability summary.

Assay	Parameter	Standard	Correct unit	n	Mean	SD	CV (%)	Min	Max	Repeatability comment
TPC	Total phenolic content	Gallic acid	mg GAE/g extract	3	21.79	0.50	2.31	21.49	22.37	Very good
TFC	Total flavonoid content	Rutin	mg RE/g extract	3	8.42	0.27	3.20	8.20	8.72	Very good
TAC	Total antioxidant capacity	Ascorbic acid	mg AAE/g extract	3	17.43	0.42	2.40	17.04	17.87	Very good

Validation of ABTS and DPPH inhibition and IC50 decisions

ABTS and DPPH inhibition percentages were recalculated from raw absorbance values using the same general equation: % inhibition = $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$. In the ABTS assay, both the extract and ascorbic acid crossed 50% inhibition within the tested range, allowing true linear interpolation of IC50 values. The extract IC50 was 185.93 µg/mL, whereas ascorbic acid showed an IC50 of 41.62 µg/mL; consequently, the extract was approximately 4.47-fold less active than ascorbic acid under the same assay conditions.

In the DPPH assay, the ascorbic acid standard reached 50% inhibition and produced a true interpolated IC50 of 52.55 µg/mL. In contrast, the extract reached only 46.61% inhibition at 1000 µg/mL. Therefore, the scientifically correct statement is IC50 > 1000 µg/mL. The calculated value of approximately 1076.29 µg/mL is an extrapolated estimate and should not be reported as a true experimental IC50.

Analytical interpretation

The dataset supports a robust TPC estimate and descriptive ABTS/DPPH response curves. TFC and TAC are useful only as preliminary screening estimates because their calibration curves were suboptimal. Their low sample CV values indicate consistent replicate readings within the same analytical run, but do not establish accuracy.

Accordingly, subsequent discussion uses TFC and TAC cautiously and avoids the terms “validated quantitative result” or “quality-control specification”. The values remain suitable for identifying broad trends and planning reanalysis, but not for fine ranking, commercial standard setting or regulatory submission.

The DPPH extract response is reported as IC50 > 1000 µg/mL. No extrapolated DPPH value is used in secondary indices.

III.2. Qualitative phytochemical screening

The qualitative phytochemical screening of the hydroethanolic extract of *C. monacantha* was designed as a preliminary chemical orientation step before quantitative phenolic/flavonoid determinations and antioxidant testing. In classical pharmacognosy and phytochemical analysis, colour reactions and frothing tests are used as presumptive assays to indicate the possible occurrence of broad metabolite families, particularly phenolic compounds, flavonoids, terpenoid-type constituents and saponins. These reactions are rapid and informative, but they remain qualitative and chemically non-specific; consequently, they should guide subsequent quantitative and chromatographic work rather than replace it (Trease and Evans, 2002; Harborne, 1998; Tiwari et al., 2011; Godlewska et al., 2023).

For the present extract, the screening results were interpreted conservatively. The visual responses were converted into the symbols -, +, ++ and +++, and then into ordinal descriptive scores only for graphical visualization. No inferential statistics were applied because these observations do not represent replicated concentration measurements. The purpose of this section is therefore to establish a coherent qualitative chemical basis for the following measurements of total phenolic content, total flavonoid content, total antioxidant capacity, ABTS radical cation scavenging activity and DPPH radical scavenging activity.

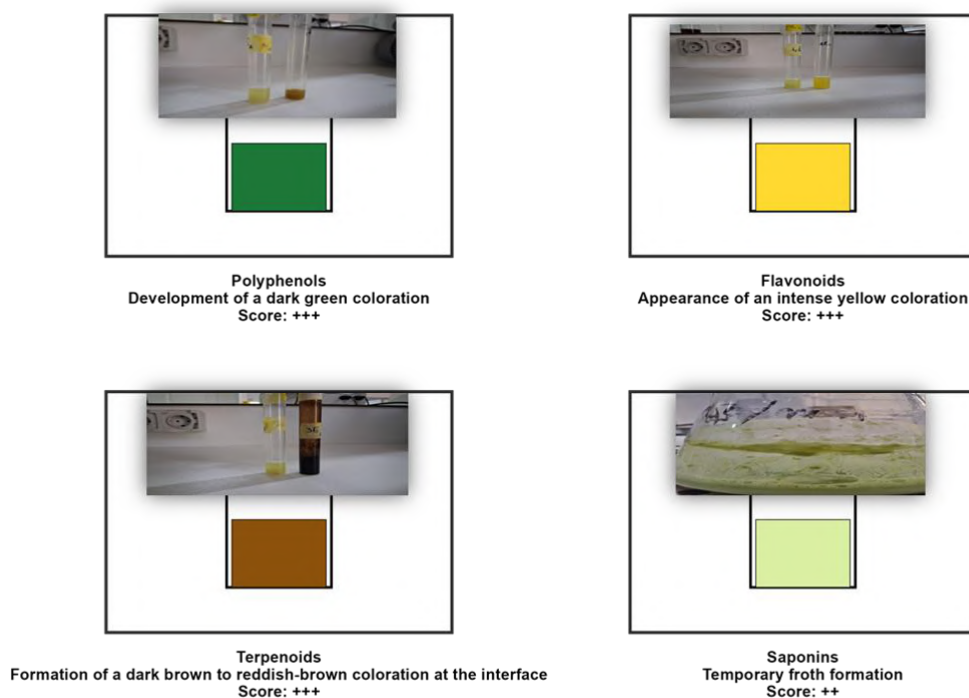


Figure 15. Visual phytochemical screening plate showing the characteristic colour reactions and froth formation observed for the hydroethanolic extract of *C. monacantha*.

The visual screening plate shows four positive qualitative responses. A dark green colour was observed in the ferric chloride reaction, which is consistent with the possible presence of phenolic hydroxyl-containing compounds capable of forming coloured complexes with iron salts. An intense yellow colour was observed under alkaline conditions, supporting the presence of flavonoid-type constituents. The

Salkowski-type reaction produced a dark brown to reddish-brown colour at the interface, which is compatible with terpenoid-type constituents. Finally, the frothing test generated temporary foam, indicating a moderate saponin-type response. Such test outcomes are consistent with the known phytochemical complexity of *C. monacantha*, for which polyphenols, flavonoid derivatives, isoflavones and triterpenoidal saponins have been reported in earlier phytochemical studies (Kamel et al., 2000; Kandil and Grace, 2001; Mhiri et al., 2020; Badawy et al., 2024).

Table 4. Phytochemical screening of the hydroethanolic extract

Constituent	Test used	Characteristic observation	Intensity score	Presence	Interpretation
Polyphenols	Ferric chloride test	Development of a dark green coloration	+++	Present	Strong positive reaction suggesting the presence of phenolic compounds able to complex ferric ions.
Flavonoids	Alkaline reagent / flavonoid colour reaction	Appearance of an intense yellow coloration	+++	Present	Strong positive reaction suggesting the presence of flavonoid-type compounds under alkaline conditions.
Terpenoids	Salkowski-type reaction	Formation of a dark brown to reddish-brown coloration at the interface	+++	Present	Strong positive reaction suggesting the presence of terpenoid-type constituents.
Saponins	Frothing test	Temporary froth formation	++	Present	Moderate positive reaction suggesting the presence of saponins with surface-active properties.

Note. The symbols indicate visual reaction intensity only. They should not be interpreted as concentrations or as quantitative abundance classes.

To avoid over-quantification, the semi-quantitative symbols were treated as an ordinal descriptive scale. This transformation is useful for heatmap construction and graphical communication, but it does not provide a numerical concentration. The scale used in this work is reported in Table 5.

Table 5. Semi-quantitative scoring scale

Symbol	Ordinal score	Meaning	Interpretation	Methodological caution
-	0	Absent	No visible characteristic reaction under the test conditions.	Qualitative result only.
+	1	Weak reaction	Slight visible reaction; possible trace or low abundance.	Qualitative result only; not a concentration.
++	2	Moderate reaction	Clear visible reaction; moderate qualitative presence.	Qualitative result only; not a concentration.
+++	3	Strong reaction	Intense visible reaction; strong qualitative presence.	Qualitative result only; not a concentration.

Note. The ordinal score was used exclusively to construct the heatmap and barplot. It was not used for hypothesis testing.

The heatmap in Figure16 summarizes the qualitative intensity profile. Polyphenols, flavonoids and terpenoids reached the strongest visual score (+++), whereas saponins showed a moderate response (++). This pattern is important because phenolic and flavonoid compounds are frequently associated with electron-donating, hydrogen-donating and metal-complexing properties, while terpenoid and saponin-type metabolites may contribute to the global bioactive signature of the extract. However, the heatmap must be read as a descriptive visualization, not as a quantitative matrix.

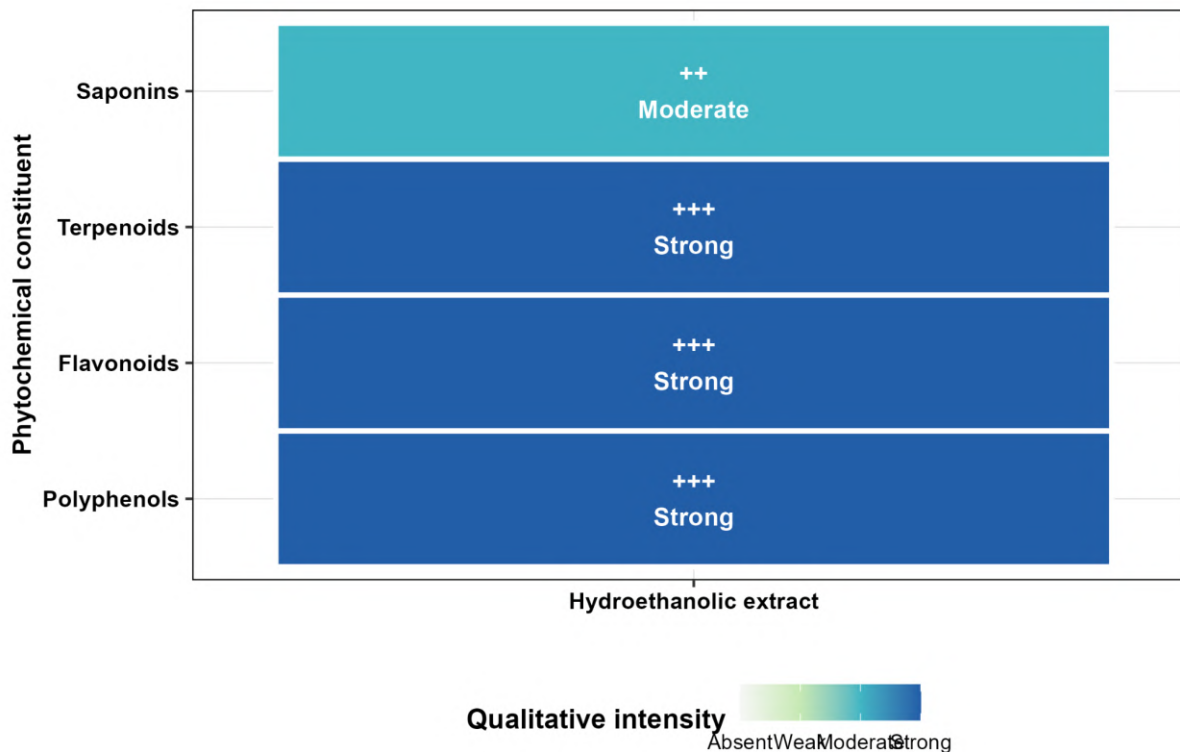


Figure16. Phytochemical intensity heatmap based on qualitative ordinal scores.

The strong positive response for polyphenols is analytically coherent with the subsequent total phenolic content assay. Ferric chloride-based reactions are among the classical rapid tests for phenolic hydroxyl groups, because phenolics may form coloured complexes with ferric ions. Nevertheless, the response is group-level and presumptive: it does not distinguish phenolic acids, flavonoids, gallotannins or other complex polyphenolic derivatives. For this reason, the qualitative result should be linked to the TPC assay and ultimately confirmed by chromatographic or mass-spectrometric profiling (Harborne, 1998; Waterhouse, 2002; Prior et al., 2005).

The intense yellow reaction attributed to flavonoid-type compounds supports the corrected interpretation of the following total flavonoid content result. This point is particularly relevant because the quantitative TFC assay was calibrated with rutin; consequently, the final quantitative result must be expressed as mg rutin equivalents per gram of extract (mg RE/g extract), not as mg quercetin equivalents. The qualitative flavonoid response is also consistent with the phytochemical literature on *C. monacantha*, including the report of polyphenolic and flavonoid-type constituents by Kandil and Grace (2001), the isolation of antioxidant isoflavones by Mhiri et al. (2020), and the more recent isolation of the isoflavone cornulacin by Badawy et al. (2024).

The terpenoid and saponin responses broaden the chemical interpretation of the extract. The Salkowski-type reaction suggests terpenoid-type constituents, whereas the frothing test suggests the presence of surface-active saponins. This interpretation is plausible in view of previous reports of triterpenoidal saponins from the aerial parts of *C. monacantha*, including several new saponin structures described by Kamel et al. (2000). Yet, both tests remain qualitative and cannot resolve molecular identity, aglycone type, sugar chain composition, or concentration. A robust phytochemical confirmation would therefore require LC-MS/MS, HPLC-DAD, UHPLC-HRMS or complementary NMR-based dereplication.

Overall, the hydroethanolic extract of *C. monacantha* showed a broad and coherent qualitative phytochemical profile. The most intense reactions were recorded for polyphenols, flavonoids and terpenoids (+++), while saponins showed a moderate response (++). These findings support the chemical rationale for the following quantitative assays and antioxidant measurements. However, they do not constitute proof of molecular identity, chemical concentration, biological efficacy or safety. The results should therefore be interpreted as a preliminary phytochemical orientation step that justifies, but does not replace, quantitative and chromatographic validation.

III.3. Total Phenolic Content (TPC)

III.3.1. Analytical rationale and methodological relevance

Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric assay, a classical spectrophotometric approach historically introduced for the colorimetric estimation of phenolics through phosphomolybdic-phosphotungstic acid reagents. Although the Folin-Ciocalteu assay is often reported as a measure of total phenolics, it is more precisely a global reducing-capacity assay in which phenolic hydroxyl groups, and potentially other reducing constituents, reduce the reagent under alkaline conditions to form a blue chromophore measured at 765 nm (Singleton and Rossi, 1965; Slinkard and Singleton, 1977; Waterhouse, 2002; Huang et al., 2005; Prior et al., 2005; Ainsworth and Gillespie, 2007; Everette et al., 2010; Blainski et al., 2013; Pérez et al., 2023). Consequently, the TPC result should be interpreted as a calibrated phenolic-equivalent index rather than as a direct molecular quantification of all individual phenolic structures.

In the present study, gallic acid was used as the reference standard and the result was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). This expression is appropriate because it preserves traceability to the calibration standard and enables descriptive comparison with published studies on *C. monacantha* and other Saharan halophytes. However, literature comparisons must remain cautious because TPC values are strongly affected by solvent polarity, extraction time, temperature, solid-to-liquid ratio, plant organ, geographical origin, drying conditions and assay implementation (Dai and Mumper, 2010; Khoddami et al., 2013; Dent et al., 2013; Lemmadi et al., 2025).

III.3.2. Gallic acid calibration curve

The gallic acid calibration curve showed excellent linearity over the working range of 0-200 µg/mL. The regression equation was $Abs = 0.009444 \times C - 0.0053$, with $R^2 = 0.9995$. This very high coefficient of determination indicates that the calibration model was suitable for converting sample absorbance into equivalent gallic acid concentration within the tested range. The slightly negative intercept is analytically acceptable and was considered in the concentration calculation by using the corrected formula $C = (Abs - intercept)/slope$; because the intercept is negative, this is equivalent to $C = (Abs + 0.0053)/0.009444$.

Table 6. Gallic acid calibration curve used for TPC determination.

Standard	Concentration (µg/mL)	Absorbance 765 nm	Regression equation	R ²	Comment
Gallic acid	0	0.0003	$Abs = 0.009444 \times C - 0.0053$	0.9995	Excellent linearity
Gallic acid	50	0.4659	$Abs = 0.009444 \times C - 0.0053$	0.9995	Excellent linearity
Gallic acid	100	0.9180	$Abs = 0.009444 \times C - 0.0053$	0.9995	Excellent linearity
Gallic acid	150	1.4340	$Abs = 0.009444 \times C - 0.0053$	0.9995	Excellent linearity
Gallic acid	200	1.8772	$Abs = 0.009444 \times C - 0.0053$	0.9995	Excellent linearity

Note. C denotes the equivalent gallic acid concentration. The high R² supports the use of the regression equation for converting the sample absorbance values into gallic acid equivalents.

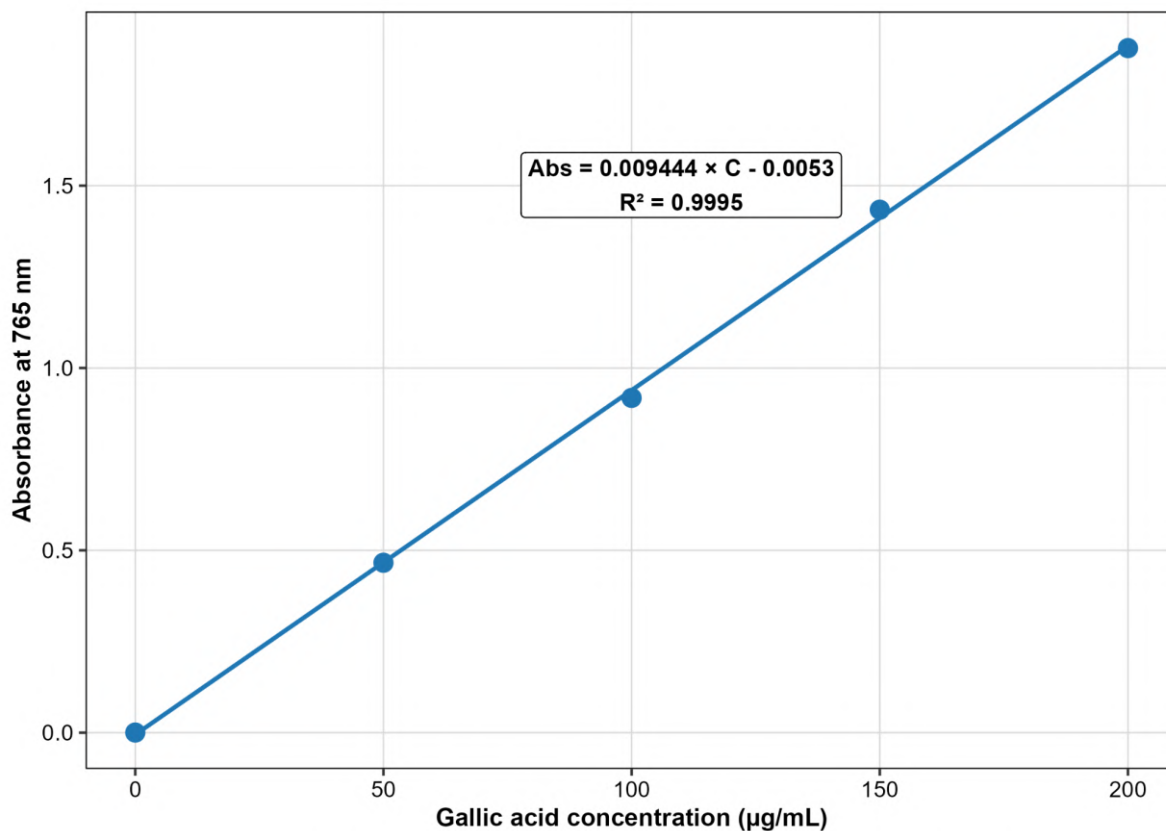


Figure 17. Gallic acid calibration curve used for total phenolic content determination.

III.3.3. Calculation of TPC from sample absorbance

The sample absorbances were converted into gallic acid-equivalent concentrations using the calibration equation. Under the adopted conditions ($V = 10$ mL, dilution factor $D = 2$ and extract mass $m = 0.1$ g), the general equation $TPC = (C \times V \times D)/m$, followed by conversion from $\mu\text{g/g}$ to mg/g , becomes numerically equivalent to $TPC (\text{mg GAE/g extract}) = 0.2 \times C$, where C is expressed in $\mu\text{g/mL}$. This calculation yielded replicate values of 22.37, 21.49 and 21.50 mg GAE/g extract, showing a narrow dispersion between the three analytical repetitions.

Table 7. TPC calculation from absorbance.

Replicate	Abs 765 nm	C_GA ($\mu\text{g/mL}$)	D	V (mL)	m (g)	TPC (mg GAE/g)
DO1	1.0510	111.85	2	10	0.1	22.37
DO2	1.0096	107.47	2	10	0.1	21.49
DO3	1.0101	107.52	2	10	0.1	21.50

Note. TPC was calculated from the gallic acid-equivalent concentration after correction for volume, dilution factor and extract mass. The final values are expressed as mg GAE/g extract.

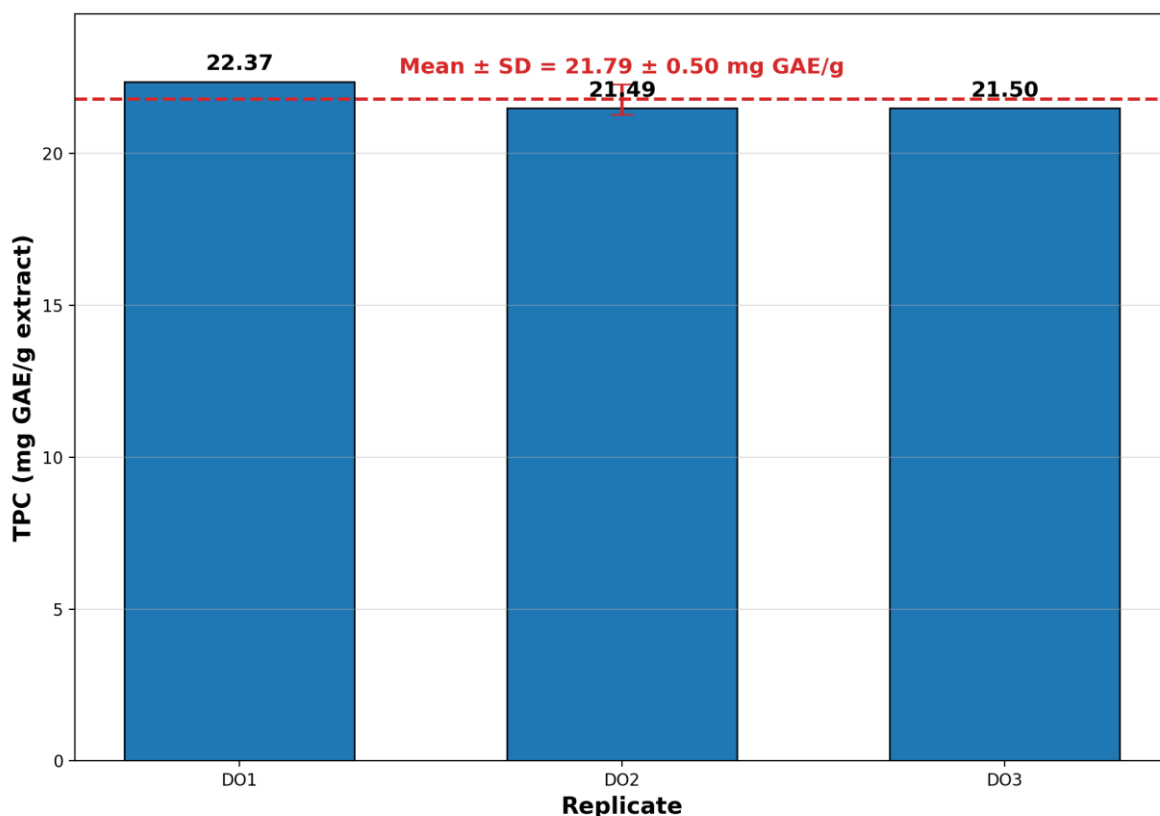


Figure 18. Total phenolic content in three analytical replicates of *C. monacantha* extract.

III.3.4. Final TPC result and analytical repeatability

The hydroethanolic extract of *C. monacantha* showed a TPC of 21.79 +/- 0.50 mg GAE/g extract, with a coefficient of variation of 2.31%. The low CV indicates very good repeatability for the triplicate analytical measurements. The optional 95% confidence interval may be reported for transparency, but it should not be overinterpreted because the number of analytical replicates was limited to n = 3.

Table 8. Final TPC result.

Parameter	n	Mean	SD	CV (%)	Unit	Interpretation
Total phenolic content	3	21.79	0.50	2.31	mg GAE/g extract	Moderate total phenolic content; lower than several published values for <i>C. monacantha</i> , probably depending on solvent, extraction method and geographical origin.

Note. The confidence interval is useful as a descriptive precision indicator but should be interpreted cautiously because n = 3.

III.3.5. Interpretation of phenolic richness

The value obtained in the present work indicates a moderate total phenolic content. This interpretation is supported by the comparison with available data on *C. monacantha*, where several reported values are higher than the present hydroethanolic extract. For example, an aqueous extract from south-eastern Algeria was reported at approximately 31.33 mg GAE/g extract, while optimized ultrasound-assisted extraction of the aerial parts with ethanol/water generated a TPC of 37.27 ug GAE/mg dry extract,

numerically equivalent to 37.27 mg GAE/g dry extract (Ali Boutlelis et al.; Lemmadi et al., 2025). A higher value of 64.03 ug GAE/mg dry extract was reported for a methanolic extract from the Grand Erg region, and a recent methanolic extract study reported 29.91 +/- 1.61 ug GAE/mg extract, equivalent to 29.91 mg GAE/g extract (Boussadia et al.; Eltamany et al., 2026).

The lower TPC observed here should therefore not be interpreted as an analytical weakness. Rather, it likely reflects differences in extraction selectivity and extraction intensity. The present extract was produced by hydroethanolic maceration using ethanol/water 50:50, whereas some literature values involve methanolic extraction or optimized ultrasound-assisted extraction, both of which may enhance phenolic recovery. Moreover, *C. monacantha* is a Saharan halophyte, and secondary metabolite accumulation may vary according to drought, salinity, soil composition, season and plant developmental stage. These factors are well recognized in medicinal plant phytochemistry and are especially relevant for desert taxa exposed to strong environmental constraints.

Table 9. Literature comparison for total phenolic content.

Reference	Origin	Plant part	Solvent/extraction	Unit	Value	Comparison
Present study	Bennacer, south-eastern Algeria	Aerial parts	Hydroethanolic extract, ethanol/water 50:50	mg GAE/g extract	21.79	Reference value
Ali Boutlelis et al.	El-Oued, south-eastern Algeria	Aerial parts	Aqueous extract	mg GAE/g extract	31.33	Higher than present study
Lemmadi et al. (2025)	Algerian Sahara	Aerial parts	Ethanolic extract, optimized UAE	mg GAE/g dry extract	37.27	Higher than present study
Boussadia et al.	Grand Erg / Algerian Sahara	Aerial parts	Methanolic extract	mg GAE/g dry extract	64.03	Higher than present study
Eltamany et al. (2026)	Egypt / metabolomic study	Aerial parts	Methanolic extract	mg GAE/g extract	29.91	Higher than present study

Note. The comparison is descriptive only. Differences may reflect solvent, extraction method, plant origin, plant part, and reporting unit. Values reported as ug/mg are numerically equivalent to mg/g.

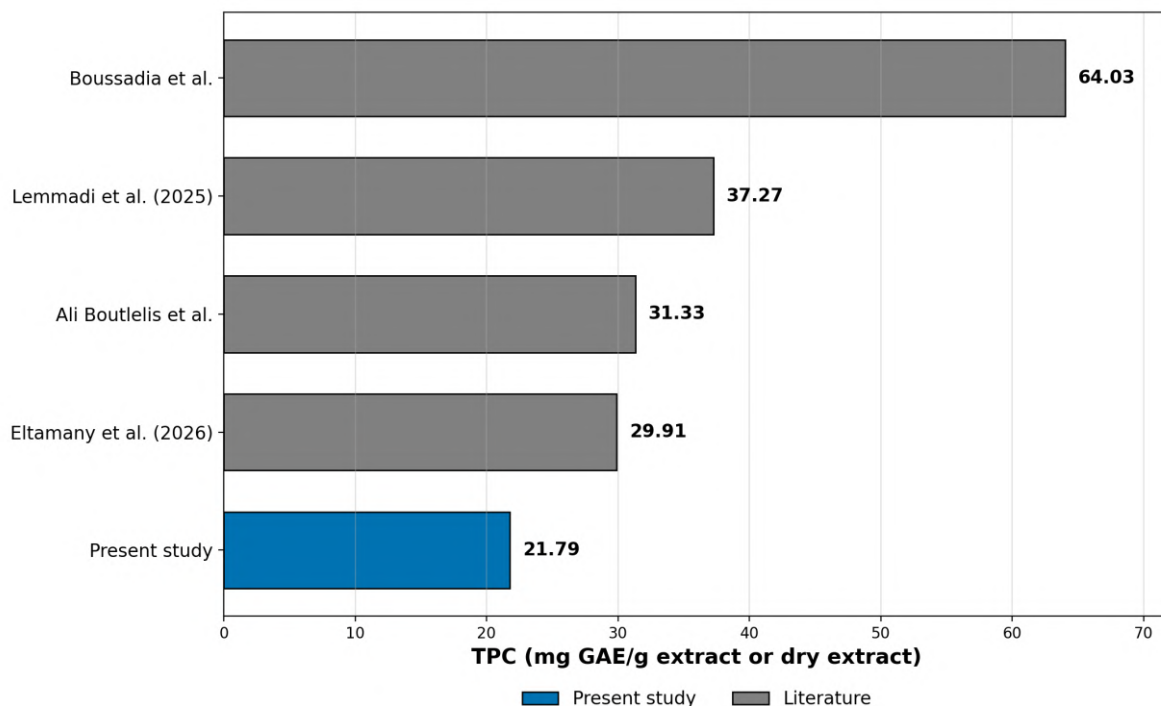


Figure 19. Literature benchmark of total phenolic content reported for *C. monacantha*.

III.3.6. Discussion within the phytochemical-antioxidant framework

Phenolic compounds are among the most important plant secondary metabolites contributing to antioxidant behavior through electron transfer, hydrogen atom transfer, radical stabilization and metal chelation mechanisms (Rice-Evans et al., 1996; Balasundram et al., 2006; Dai and Mumper, 2010; Apak et al., 2016; Gulcin, 2020). In this context, the moderate TPC of *C. monacantha* is chemically coherent with the qualitative ferric chloride screening, which indicated a strong positive reaction for phenolic-type compounds, and with the later antioxidant profile of the extract. Nevertheless, because the Folin-Ciocalteu assay is not specific to individual phenolics and does not identify molecular structures, the result should be considered an integrated phenolic-equivalent index that requires chromatographic confirmation by HPLC-DAD, LC-MS/MS or related metabolomic approaches in future work (Khoddami et al., 2013; Zeb, 2021; Lemmadi et al., 2025; Eltamany et al., 2026).

Overall, the TPC result supports the presence of a moderate phenolic reservoir in the hydroethanolic extract of *C. monacantha*. It provides a chemically plausible basis for the antioxidant assays, but it does not alone prove biological efficacy, hepatoprotective activity or safety. Its main value in this results-and-discussion chapter is therefore to establish a calibrated, reproducible and literature-positioned phenolic index that can be integrated with TFC, TAC, ABTS and DPPH results.

III.4. Total flavonoid content (TFC)

The total flavonoid content (TFC) of the hydroethanolic extract of *C. monacantha* Delile was determined using an aluminium chloride colorimetric approach and a rutin-based calibration curve. This section is analytically important because flavonoid assays are standard-dependent: the equivalent unit must reflect the compound used to build the calibration curve. Therefore, because rutin was used as the reference standard in the validated calculation file, the result must be expressed as milligrams of rutin equivalents per gram of extract (mg RE/g extract), not as milligrams of quercetin equivalents per gram of extract (mg QE/g extract). This distinction is essential for analytical traceability, inter-study comparison and scientific reporting of plant flavonoid indices.

Colorimetric flavonoid assays are classical tools in pharmacognosy and food phytochemistry, especially when the objective is to estimate a global flavonoid index rather than identify individual molecules. The aluminium chloride complexation principle is generally associated with the formation of coloured complexes between aluminium ions and flavonoid hydroxyl/keto groups, but the response depends on the flavonoid structure and on the selected standard. Consequently, rutin-, quercetin- and catechin-equivalent values are not chemically interchangeable, even when they are expressed in the same dimensional unit of mg/g (Bahorun et al., 1996; Zhishen et al., 1999; Chang et al., 2002; Djeridane et al., 2006; Stankovic, 2011; Orsavova et al., 2023).

The calibration curve used for this TFC determination was generated with rutin over the range 0-40 µg/mL, with the regression equation $Abs = 0.0042 \times C - 0.0138$ and $R^2 = 0.9595$ (Table 10; Figure 20). This linearity was sufficient for preliminary calculation, but it remains below the level expected for a fully validated analytical method. In future work, the calibration should be repeated with more calibration points, independent standard preparations and replicate absorbance readings in order to improve linearity, evaluate residuals and document the analytical performance of the method in accordance with general validation principles (ICH Q2(R2), 2023; Eurachem, 2014).

Table 10. Rutin calibration curve used for total flavonoid content determination.

Standard	Concentration (µg/mL)	Absorbance at 430 nm	Regression equation	R2	Wavelength	Range	Comment
Rutin	0	0.0000	$Abs = 0.0042 \times C - 0.0138$	0.9595	430 nm	0-40 µg/mL	Moderate linearity; improve in future validation
Rutin	10	0.0282	$Abs = 0.0042 \times C - 0.0138$	0.9595	430 nm	0-40 µg/mL	Moderate linearity; improve in future validation
Rutin	20	0.0702	$Abs = 0.0042 \times C - 0.0138$	0.9595	430 nm	0-40 µg/mL	Moderate linearity; improve in future validation
Rutin	30	0.1122	$Abs = 0.0042 \times C - 0.0138$	0.9595	430 nm	0-40 µg/mL	Moderate linearity; improve in future validation
Rutin	40	0.1542	$Abs = 0.0042 \times C - 0.0138$	0.9595	430 nm	0-40 µg/mL	Moderate linearity; improve in future validation

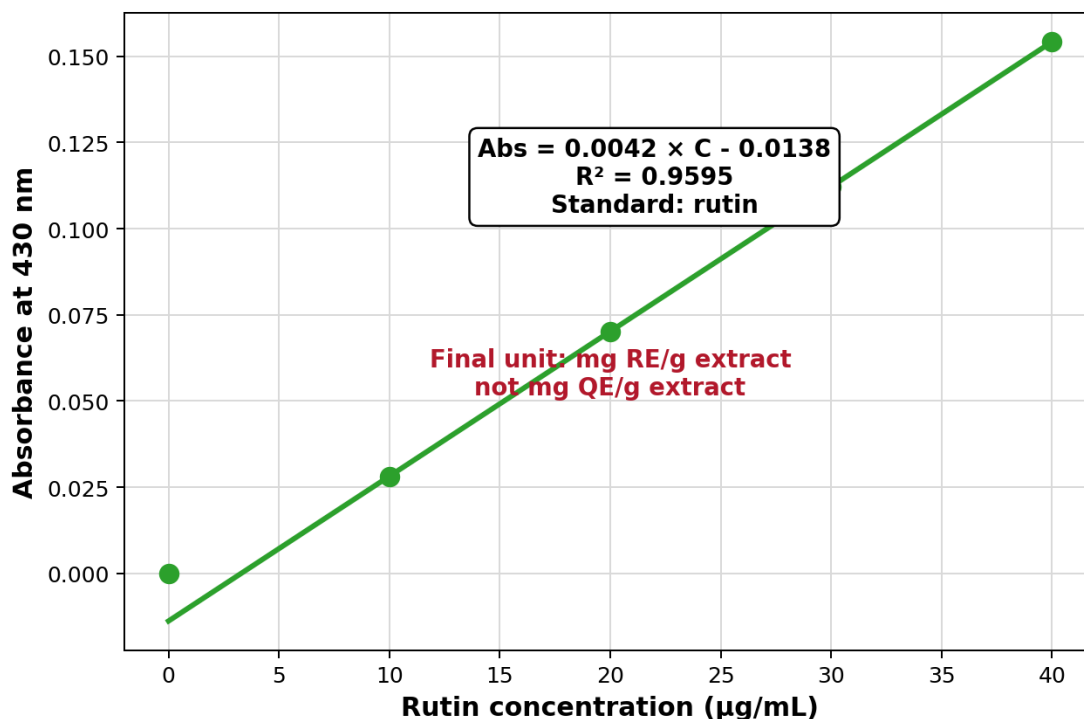


Figure 20. Rutin calibration curve used for total flavonoid content determination.

The equivalent rutin concentration of each replicate was calculated from the calibration equation by rearranging the regression model as $C = (\text{Abs} - \text{intercept}) / \text{slope}$. Because the intercept is negative, the operational expression becomes $C = (\text{Abs} + 0.0138) / 0.0042$. Under the adopted experimental conditions ($V = 10$ mL, dilution factor $D = 2$, and extract mass $m = 0.1$ g), the final expression is $\text{TFC} = (C \times V \times D) / m$, followed by conversion from $\mu\text{g/g}$ to mg/g . Numerically, this is equivalent to $\text{TFC (mg RE/g extract)} = 0.2 \times C$.

The three replicate absorbances produced TFC values of 8.35, 8.20 and 8.72 mg RE/g extract (Table 11; Figure 21). The final validated result was 8.42 ± 0.27 mg RE/g extract, with a coefficient of variation of 3.20% (Table 12). This low CV indicates good analytical repeatability for the triplicate measurements. The result should be interpreted as a moderate flavonoid content in the hydroethanolic extract, consistent with the qualitative phytochemical screening that showed a strong positive flavonoid reaction.

Table 11. TFC calculation from absorbance.

Replicate	Absorbance at 430 nm	Extract weight (g)	Volume (mL)	Dilution factor	Equivalent rutin C ($\mu\text{g/mL}$)	TFC ($\mu\text{g RE/g extract}$)	TFC (mg RE/g extract)
DO1	0.1615	0.1	10	2	41.74	8,347.62	8.35
DO2	0.1583	0.1	10	2	40.98	8,195.24	8.20
DO3	0.1693	0.1	10	2	43.60	8,719.05	8.72

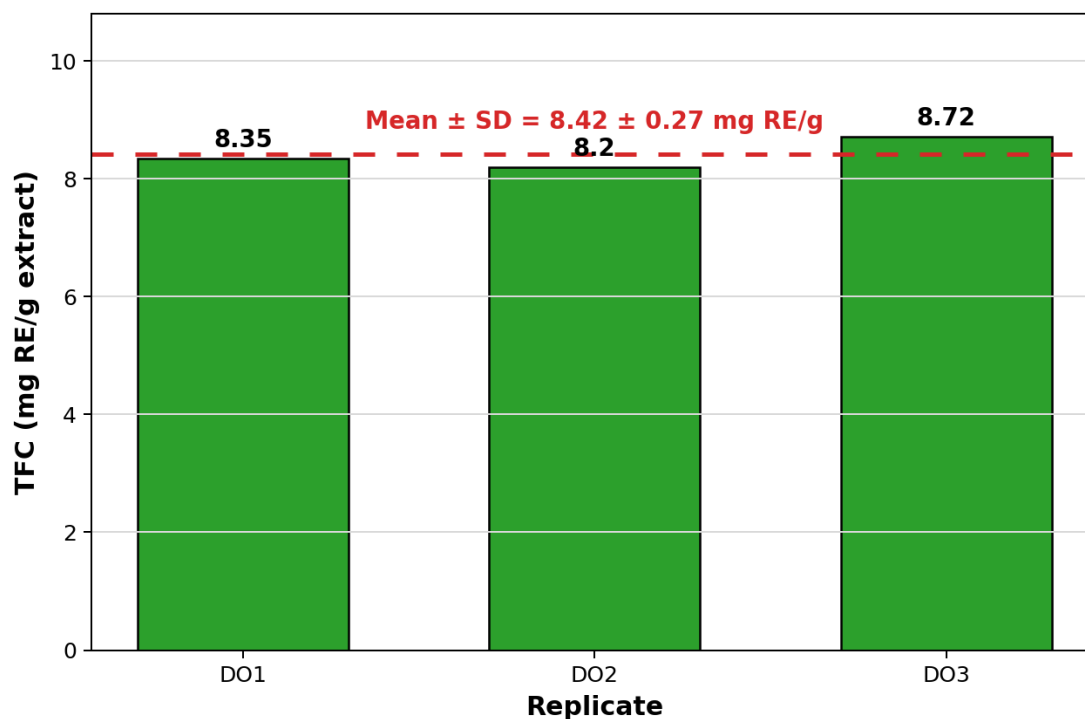


Figure 21. Total flavonoid content in three analytical replicates of *C. monacantha* extract.

Table 12. Final TFC result and unit correction.

Parameter	Standard used	Incorrect unit to avoid	Correct unit	n	Mean	SD	CV (%)	Final result	Interpretation
Total flavonoid content	Rutin	mg QE/g extract	mg RE/g extract	3	8.42	0.27	3.20	8.42 +/- 0.27 mg RE/g extract	Moderate flavonoid content with good analytical repeatability

To relate flavonoid abundance to the previously established phenolic index, a flavonoid contribution ratio (FCR) was calculated as TFC/TPC. Using TFC = 8.42 mg RE/g extract and TPC = 21.79 mg GAE/g extract, FCR = 0.386, corresponding to approximately 38.6% (Figure 22). This index is useful as a descriptive integrative indicator, but it must not be interpreted as a true molecular proportion because TFC and TPC are expressed against different calibration standards: rutin equivalents for TFC and gallic acid equivalents for TPC.

The comparison with literature must also be standard-aware. Lemmadi et al. (2025) reported a flavonoid value expressed as quercetin equivalents under optimized extraction conditions, whereas Eltamany et al. (2026) reported a rutin-equivalent value for a methanolic extract. The present result is therefore most directly comparable to studies using rutin equivalents, while quercetin-equivalent data provide only a partial benchmark. In addition, studies reporting isolated flavonoids or isoflavones from *C. monacantha*, such as Kandil and Grace (2001) and Badawy et al. (2024), support the flavonoid chemical relevance of the species but do not provide directly comparable total flavonoid contents.

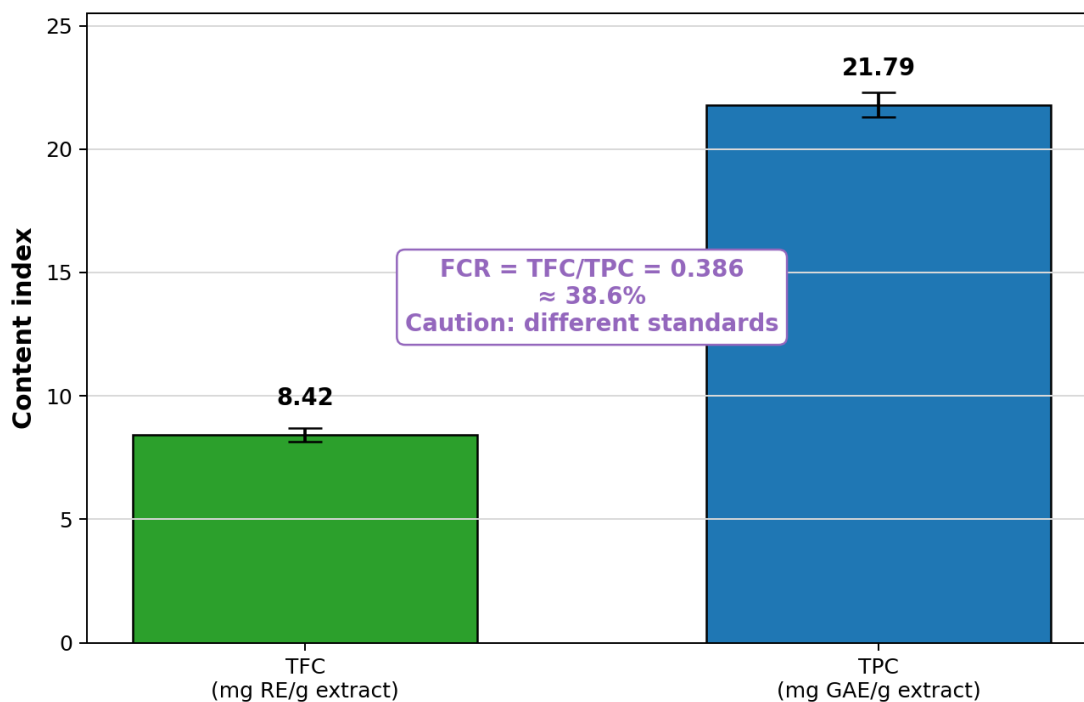


Figure 22. Relative contribution of flavonoid content to total phenolic content; interpretation is cautious because different standards were used.

Table 13. Literature comparison for flavonoids.

Reference	Standard used	Unit	Reported value	Comparability with present result	Scientific comment
Present study	Rutin	mg RE/g extract	8.42	Direct result	Correctly expressed as mg RE/g extract
Lemmadi et al. (2025)	Quercetin	mg QE/g dry extract	7.21	Partial; different standard	Reported in quercetin equivalents; compare cautiously
Eltamany et al. (2026)	Rutin	mg RE/g extract	6.30	More comparable	Reported in rutin equivalents; slightly lower than present result
Kandil & Grace (2001)	Not a total flavonoid assay	Isolated flavonoids identified	Not applicable	Not quantitatively comparable	Supports presence of flavonoid-type compounds
Badawy et al. (2024)	Not a total flavonoid assay	Isolated isoflavone identified	Not applicable	Not quantitatively comparable	Supports phytochemical diversity of <i>C. monacantha</i>

Overall, the hydroethanolic extract of *C. monacantha* exhibited a validated TFC of 8.42 +/- 0.27 mg RE/g extract with a CV of 3.20%, indicating good repeatability of the triplicate measurements. The unit correction from QE to RE is not merely cosmetic; it is required because the calibration curve was based on rutin. Reporting the value as mg QE/g extract would imply the use of quercetin as the standard and would therefore be analytically incorrect.

From a phytochemical perspective, the measured flavonoid index complements the qualitative screening and the TPC result. The flavonoid fraction appears to represent a substantial part of the global phenolic signal, as suggested by the FCR value of 0.386. Nevertheless, this index should be used as a cautious descriptive ratio rather than as a stoichiometric estimate. The moderate TFC value is compatible with the broader phytochemical profile of *C. monacantha*, for which flavonols, flavones, isoflavones and phenolic derivatives have been reported in earlier and recent phytochemical investigations.

The scientific implication is that the hydroethanolic extract contains a measurable flavonoid-related reducing/complexing pool that may contribute to antioxidant behaviour, but total flavonoid content alone cannot identify the specific active molecules. Chromatographic profiling by HPLC-DAD, LC-MS/MS or LC-HRMS would be required to determine whether the TFC signal is dominated by rutin-like compounds, quercetin derivatives, luteolin glycosides, isoflavones or other phenolic constituents.

III.5. Total Antioxidant Capacity (TAC)

The phosphomolybdenum total antioxidant capacity (TAC) assay was used to estimate the global reducing capacity of the hydroethanolic extract of *C. monacantha* Delile. In this electron-transfer-based assay, antioxidant constituents reduce molybdenum (VI) to molybdenum(V) under acidic conditions, generating a green phosphate/Mo(V) complex that is quantified spectrophotometrically. Because complex plant extracts contain multiple reducing constituents, the TAC value should be interpreted as an integrated reducing-capacity index rather than as the concentration of one specific antioxidant molecule.

The present TAC calculation was based on an ascorbic acid calibration curve measured at 695 nm, and the results were consequently expressed as milligrams of ascorbic acid equivalents per gram of extract (mg AAE/g extract). This expression is analytically appropriate because the reference compound used for calibration was ascorbic acid. The validated result was 17.43 ± 0.42 mg AAE/g extract, with a coefficient of variation (CV) of 2.40%, indicating good repeatability among the three analytical replicates. Nevertheless, the calibration coefficient of determination was $R^2 = 0.9696$, which supports preliminary interpretation but remains below the level generally expected for a fully validated quantitative analytical procedure.

Table 14. Ascorbic acid calibration curve for TAC.

Standard	Concentration (µg/mL)	Absorbance at 695 nm	Regression equation	R ²	Calibration comment
Ascorbic acid	0	0.000	$\text{Abs} = 0.001203 \times C - 0.006476$	0.9696	Acceptable for preliminary work; improvement required for full validation
Ascorbic acid	20	0.019	$\text{Abs} = 0.001203 \times C - 0.006476$	0.9696	Acceptable for preliminary work; improvement required for full validation
Ascorbic acid	40	0.034	$\text{Abs} = 0.001203 \times C - 0.006476$	0.9696	Acceptable for preliminary work; improvement required for full validation
Ascorbic acid	60	0.054	$\text{Abs} = 0.001203 \times C - 0.006476$	0.9696	Acceptable for preliminary work; improvement required for full validation
Ascorbic acid	80	0.098	$\text{Abs} = 0.001203 \times C - 0.006476$	0.9696	Acceptable for preliminary work; improvement required for full validation
Ascorbic acid	100	0.117	$\text{Abs} = 0.001203 \times C - 0.006476$	0.9696	Acceptable for preliminary work; improvement required for full validation

The calibration curve (Table 14; Figure 23) covered the concentration range 0–100 µg/mL. The regression equation used for sample conversion was $\text{Abs} = 0.001203 \times C - 0.006476$. Accordingly, the equivalent ascorbic acid concentration was calculated using $C = (\text{Abs} - \text{intercept})/\text{slope}$. The non-zero negative intercept was retained in the calculation because it derives from the fitted calibration equation and corrects the relationship between absorbance and standard concentration within the measured range. The R^2 value of 0.9696 indicates a usable relationship for preliminary screening, but it also signals dispersion around the fitted line and therefore justifies repeating the calibration in future work with additional points, replicate standards, and strict blank control.

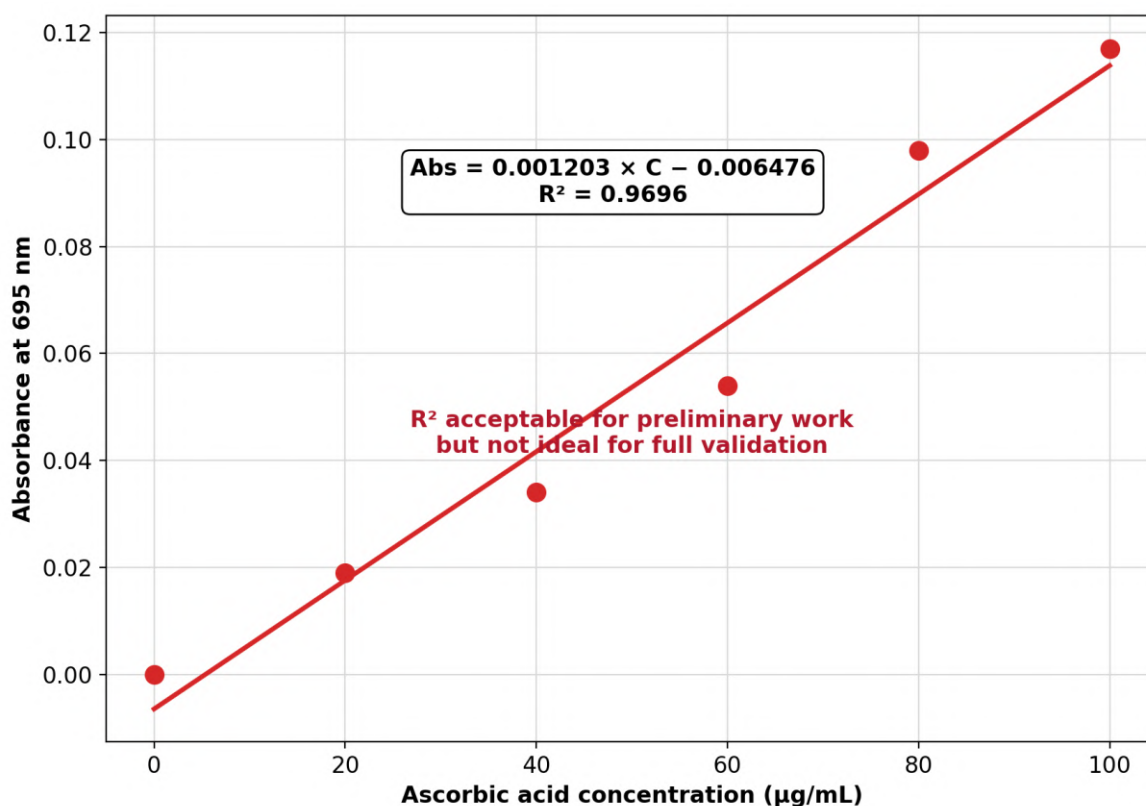


Figure 23. Ascorbic acid calibration curve used for total antioxidant capacity determination.

Table 15. TAC calculation table.

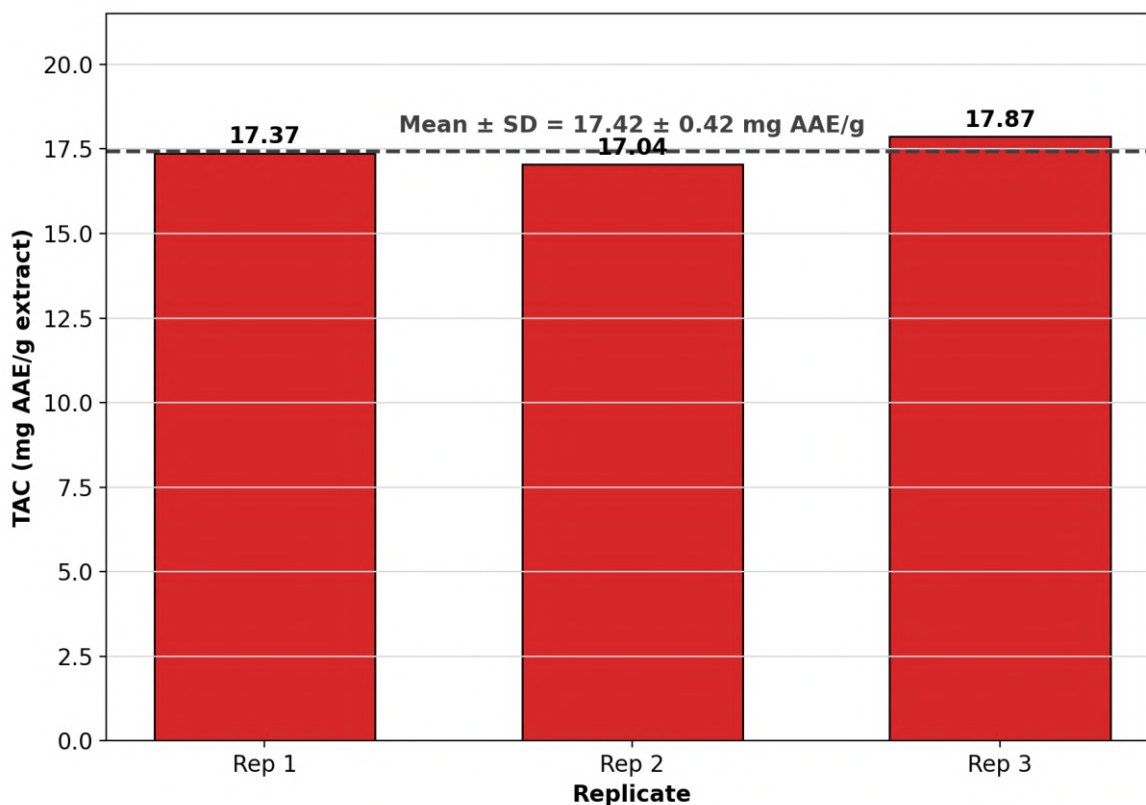
Replicate	Absorbance 695 nm	Equivalent ascorbic acid concentration (µg/mL)	Dilution factor	Final TAC (mg AAE/g extract)
Rep 1	0.098	86.85	2	17.37
Rep 2	0.096	85.18	2	17.04
Rep 3	0.101	89.34	2	17.87

For each replicate, TAC was calculated according to $TAC = (C \times V \times D)/m$ followed by conversion from micrograms per gram to milligrams per gram. Under the adopted conditions ($V = 10$ mL, $D = 2$, and $m = 0.1$ g), the final TAC in mg AAE/g extract is equivalent to $0.2 \times C$, where C is the equivalent ascorbic acid concentration in µg/mL. The three replicate values were 17.37, 17.04, and 17.87 mg AAE/g extract (Table 15).

Table 16. Final TAC result.

Item	Value
Parameter	Total antioxidant capacity
Method	Phosphomolybdenum assay
Reference standard	Ascorbic acid
Correct unit	mg AAE/g extract
Number of analytical replicates	3
Mean $\pm$ SD	17.43 $\pm$ 0.42 mg AAE/g extract
Coefficient of variation	2.40%
Calibration R ²	0.9696
Interpretation	Moderate TAC with good analytical repeatability; the calibration is acceptable for preliminary work but should be improved for full analytical validation.

The low CV value (2.40%) supports good repeatability at the level of sample measurement. This repeatability should not be confused with full method validation: it describes dispersion among the three extract replicates, whereas validation would additionally require systematic assessment of linearity, accuracy, precision, robustness, limit of detection and limit of quantification. The TAC value therefore provides a reliable preliminary estimate of reducing capacity, but future analytical standardization should improve the calibration quality before using the method for strict quality-control specifications.

**Figure 24.** Total antioxidant capacity in three analytical replicates of *C. monacantha* extract.

Integration with TPC and TFC: The TAC result complements the previously quantified total phenolic and flavonoid indices. The global TPC–TFC–TAC representation (Figure 25) should be read as a descriptive synthesis of the extract profile rather than as a direct molecular comparison. TPC is expressed as gallic acid equivalents, TFC as rutin equivalents, and TAC as ascorbic acid equivalents. These three metrics are analytically useful but chemically non-equivalent. Thus, the figure highlights the coexistence of moderate phenolic richness, moderate flavonoid content and moderate reducing capacity, while avoiding the claim that the three values quantify the same class of molecules.

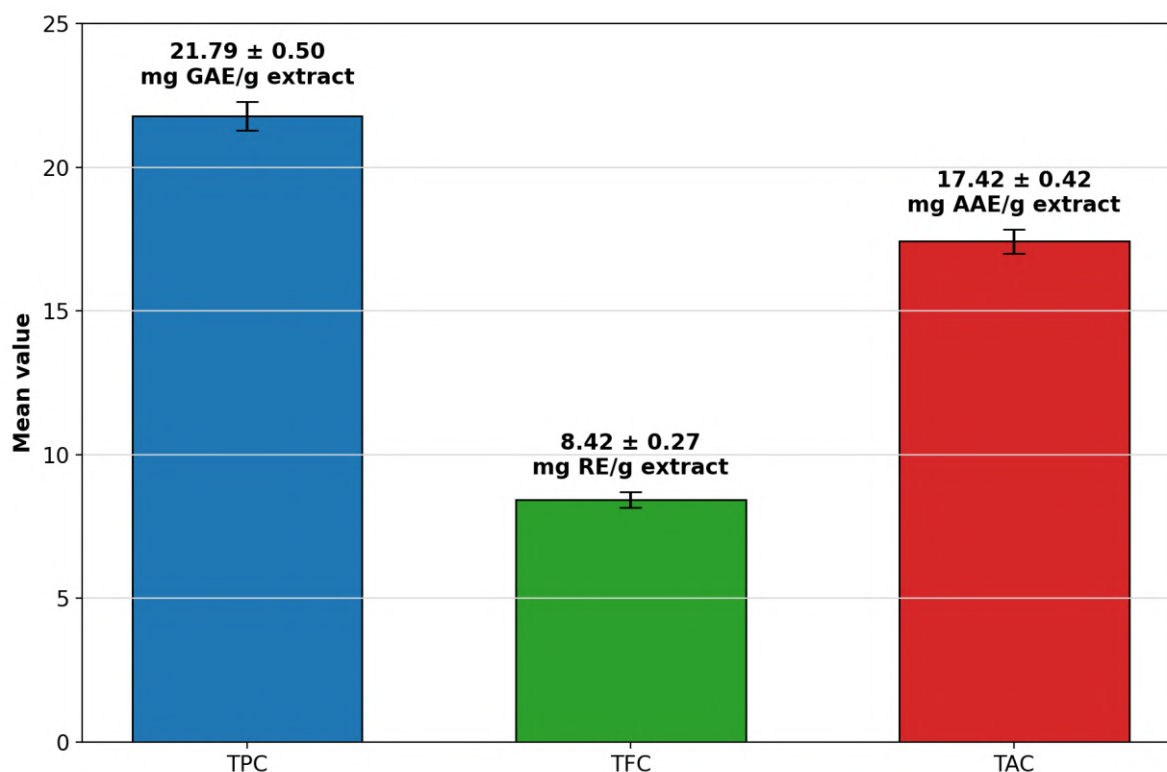


Figure 25. Global bioactive composition and reducing capacity of *C. monacantha* extract; units are based on different reference standards.

Overall, the hydroethanolic extract of *C. monacantha* displayed a moderate total antioxidant capacity. This observation is coherent with the presence of phenolic and flavonoid constituents and with the positive qualitative phytochemical screening. However, the phosphomolybdenum assay is based on a reducing reaction and does not directly establish radical-scavenging behavior, cellular antioxidant protection or hepatoprotective efficacy. The TAC result should therefore be discussed together with ABTS and DPPH data, and later with cell-based oxidative-stress models if the extract is to be positioned as a candidate botanical ingredient for liver-function support.

III.6. ABTS Radical Cation Scavenging Activity

III.6.1. Scientific rationale of the ABTS assay

The ABTS radical cation decolorization assay was used as a complementary electron-transfer based antioxidant test to evaluate the ability of the hydroethanolic extract of *C. monacantha* to reduce the pre-formed blue-green ABTS radical cation (ABTS^{•+}). The assay is historically rooted in the work of Miller et al. (1993) and was later standardized and widely popularized by Re et al. (1999). Its usefulness lies in its applicability to both hydrophilic and lipophilic antioxidant systems and in the rapid spectrophotometric monitoring of radical cation quenching at 734 nm. Because the antioxidant behavior of complex plant extracts depends on solvent polarity, reaction medium, reaction time, radical system, and standard used, ABTS should not be interpreted as a stand-alone measure of antioxidant capacity, but rather as one component of a multi-assay antioxidant profile (Arts et al., 2004; Huang et al., 2005; Prior et al., 2005; Apak et al., 2016; Gulcin, 2020).

In the present analysis, corrected inhibition percentages were calculated directly from the raw absorbance values according to the equation: inhibition (%) = [(A_{control} - A_{sample}) / A_{control}] x 100. The IC₅₀ values were then calculated by linear interpolation only because the 50% inhibition threshold was experimentally bracketed by two measured concentrations for both the extract and the reference antioxidant. This distinction is methodologically important because interpolated IC₅₀ values are more reliable than extrapolated values, particularly in antioxidant dose-response studies (Sebaugh, 2011; Ritz et al., 2015).

III.6.2. Control absorbance and analytical caution

The mean experimental control absorbance was approximately 0.322 for both the extract and the ascorbic acid series. This value is lower than the classical ABTS recommendation in which the working radical solution is often diluted to about 0.70 ± 0.02 at 734 nm before use. Therefore, the present inhibition percentages were not forced against a theoretical control value; instead, they were calculated using the actual experimental control absorbance. This choice preserves analytical transparency and avoids artificially modifying the inhibition profile. The lower control absorbance should be considered a methodological limitation when comparing the present IC₅₀ values with literature values obtained under classical ABTS conditions.

III.6.3. Corrected ABTS inhibition profile

Table 17. Corrected ABTS inhibition percentages of the hydroethanolic extract and ascorbic acid.

Sample	Concentration (µg/mL)	Mean absorbance	SD absorbance	CV absorbance (%)	Corrected inhibition (%)
Cornulaca extract	0	0.3217	0.0006	0.18	0.00
Cornulaca extract	200	0.1487	0.0021	1.40	53.78
Cornulaca extract	400	0.1007	0.0021	2.07	68.70
Cornulaca extract	600	0.0847	0.0015	1.80	73.68
Cornulaca extract	800	0.0373	0.0051	13.75	88.39
Cornulaca extract	1000	0.0157	0.0021	13.29	95.13
Ascorbic acid	0	0.3217	0.0006	0.18	0.00
Ascorbic acid	20	0.2040	0.0125	6.14	36.58

Ascorbic acid	40	0.1687	0.0031	1.81	47.56
Ascorbic acid	60	0.0720	0.0010	1.39	77.62
Ascorbic acid	80	0.0110	0.0046	41.66	96.58
Ascorbic acid	100	0.0037	0.0025	68.63	98.86

The extract showed a concentration-dependent decrease in ABTS absorbance, corresponding to a progressive increase in corrected inhibition from 53.78% at 200 $\mu\text{g/mL}$ to 95.13% at 1000 $\mu\text{g/mL}$. The reference antioxidant, ascorbic acid, showed a sharper response, increasing from 36.58% inhibition at 20 $\mu\text{g/mL}$ to 98.86% at 100 $\mu\text{g/mL}$. This confirms that the extract contains constituents capable of reducing $\text{ABTS}^{\bullet+}$, but with lower potency than the reference antioxidant under the same experimental conditions.

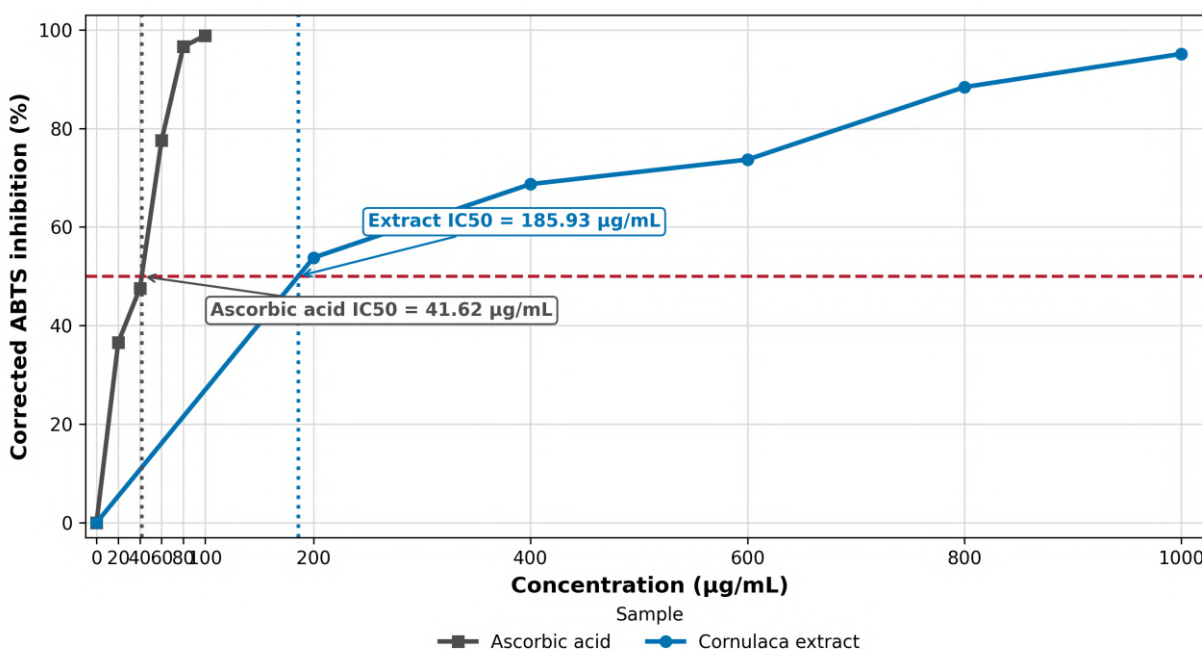


Figure 26. ABTS dose-response curve of the hydroethanolic extract of *C. monacantha* and ascorbic acid.

III.6.4. IC₅₀ interpolation and antioxidant potency

Table 18. ABTS IC₅₀ interpolation table.

Sample	C1 ($\mu\text{g/mL}$)	I1 (%)	C2 ($\mu\text{g/mL}$)	I2 (%)	IC ₅₀ ($\mu\text{g/mL}$)	IC ₅₀ (mg/mL)	Interpretation
Ascorbic acid	40	47.56	60	77.62	41.62	0.0416	True IC ₅₀ obtained by linear interpolation because 50% inhibition was reached within the tested concentration range.
Cornulaca extract	0	0.00	200	53.78	185.93	0.1859	True IC ₅₀ obtained by linear interpolation because 50% inhibition was reached within the tested concentration range.

The calculated IC₅₀ of the hydroethanolic extract was 185.93 $\mu\text{g/mL}$, whereas the IC₅₀ of ascorbic acid was 41.62 $\mu\text{g/mL}$. In both cases, the IC₅₀ values are true interpolated values because 50% inhibition was located within the experimentally tested concentration range. For the extract, the 50% threshold was bracketed between 0% inhibition at 0 $\mu\text{g/mL}$ and 53.78% inhibition at 200 $\mu\text{g/mL}$; for ascorbic acid, it was bracketed between 47.56% at 40 $\mu\text{g/mL}$ and 77.62% at 60 $\mu\text{g/mL}$.

Table 19. ABTS potency comparison between the extract and ascorbic acid.

Sample	IC50 (µg/mL)	IC50 (mg/mL)	Relative activity	Interpretation
Cornulaca extract	185.93	0.1859	Approximately 4.47-fold less active than ascorbic acid	Lower radical scavenging potency than ascorbic acid under the same experimental conditions.
Ascorbic acid	41.62	0.0416	Reference antioxidant	Stronger radical scavenging activity; lower IC50 indicates higher potency.

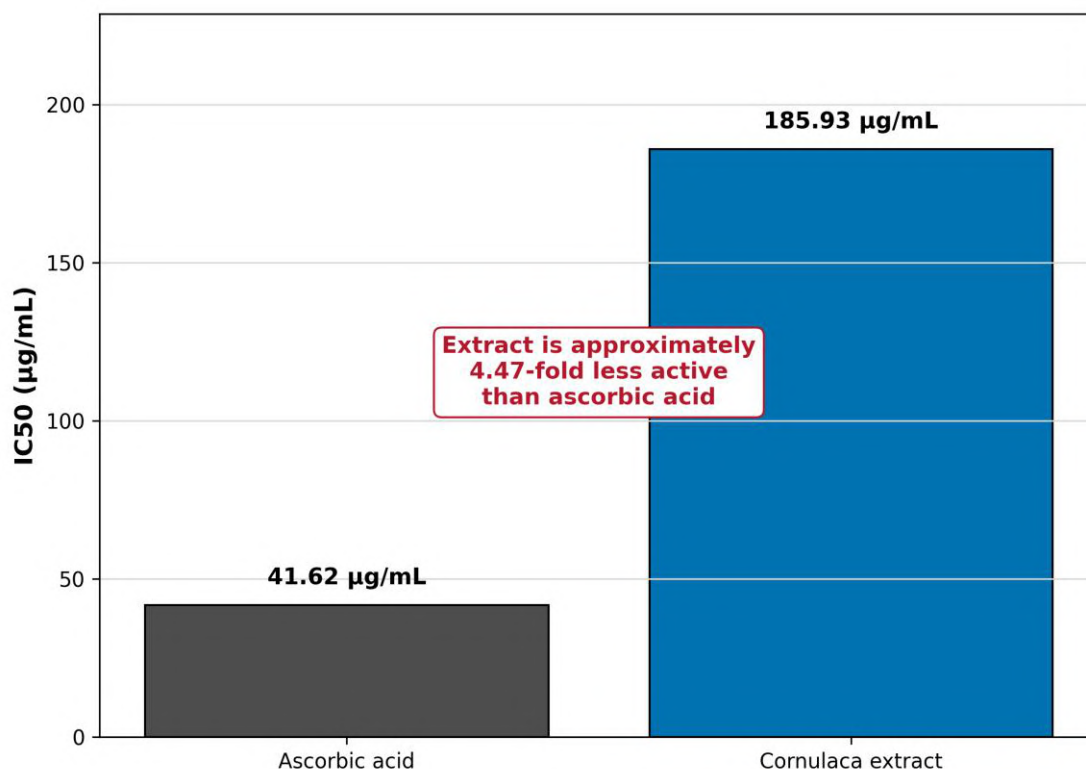


Figure 27. ABTS IC50 comparison chart. Lower IC50 indicates stronger radical cation scavenging activity.

The potency ratio, calculated as $\text{IC}_{50} \text{ extract} / \text{IC}_{50} \text{ ascorbic acid}$, was $185.93 / 41.62 = 4.47$. Therefore, the extract was approximately 4.47-fold less active than ascorbic acid in the ABTS system. This result should not be interpreted as weak antioxidant capacity in absolute terms; rather, it indicates that the extract is less potent than a pure low-molecular-weight reference antioxidant while still displaying a marked ABTS radical cation scavenging response.

Discussion and biological interpretation: The ABTS result is consistent with the qualitative and quantitative phytochemical profile of the extract. The strong qualitative detection of polyphenols and flavonoids, together with the moderate total phenolic and flavonoid contents reported in the preceding subsections, provides a plausible chemical basis for the observed radical cation scavenging activity. Phenolic hydroxyl groups and conjugated structures can donate electrons or hydrogen atoms to radical species, leading to chromophore bleaching in ABTS and related assays. The relationship between phenolic

structure and antioxidant behavior has been extensively described for flavonoids and phenolic acids (Rice-Evans et al., 1996; Pietta, 2000; Heim et al., 2002; Khoddami et al., 2013).

However, the ABTS assay remains a chemical screening method. It does not identify the compounds responsible for the activity, does not distinguish between additive and synergistic effects, and does not demonstrate cellular antioxidant protection. The observed IC₅₀ should therefore be interpreted as an in vitro radical cation scavenging descriptor rather than direct evidence of biological or hepatoprotective efficacy. Future LC-MS/MS profiling and cell-based oxidative stress assays are required to link the ABTS response to specific metabolites and biologically relevant mechanisms.

A further limitation concerns the control absorbance. Classical ABTS protocols often adjust the working ABTS•⁺ solution near 0.70 ± 0.02 at 734 nm; the present experimental control was lower, approximately 0.322. Although the inhibition calculation remains internally valid because it used the actual control absorbance, direct comparison with external studies must be cautious. This methodological transparency is essential because ABTS values are sensitive to radical preparation, dilution, incubation time, solvent system, wavelength, and reference standard (Re et al., 1999; Arts et al., 2004; Prior et al., 2005; Zhen et al., 2016).

Overall, the hydroethanolic extract of *C. monacantha* exhibited a clear concentration-dependent ABTS radical cation scavenging activity with a true interpolated IC₅₀ of 185.93 µg/mL. Ascorbic acid showed a lower IC₅₀ of 41.62 µg/mL, indicating stronger antioxidant potency. The extract was therefore approximately 4.47-fold less active than ascorbic acid under the same assay conditions. These results support the antioxidant potential of the extract, particularly in the ABTS system, while maintaining the necessary methodological caution regarding control absorbance and the chemical nature of the assay.

III.7. DPPH radical scavenging activity

III.7.1. Analytical rationale and interpretative boundary

The DPPH radical scavenging assay was used as a complementary chemical antioxidant test to evaluate the ability of the hydroethanolic extract of *C. monacantha* Delile to reduce the stable 2,2-diphenyl-1-picrylhydrazyl radical. The assay is historically rooted in the classical free-radical approach introduced by Blois and later standardized for food and plant antioxidant evaluation by Brand-Williams and co-workers. Mechanistically, the decrease in absorbance at 517 nm reflects the reduction of the purple DPPH radical into a less colored reduced form through electron-transfer and/or hydrogen-atom-transfer processes. However, the DPPH assay is strongly influenced by solvent, reaction time, steric accessibility of antioxidants, hydrophobicity of compounds and the chemical nature of the extract; therefore, it should not be interpreted as a complete measure of biological antioxidant efficacy by itself (Blois, 1958; Brand-Williams et al., 1995; Molyneux, 2004; Huang et al., 2005; Prior et al., 2005; Kedare and Singh, 2011; Apak et al., 2016; Gulcin, 2020; Gulcin, 2023).

In the present work, the DPPH inhibition percentages were recalculated from the raw absorbance values using the corrected equation: inhibition (%) = [(Acontrol - Asample) / Acontrol] × 100. This correction is essential because IC₅₀ determination depends directly on the accuracy of the inhibition curve. The critical analytical point is that the *Cornulaca* extract did not reach 50% inhibition within the tested concentration range. Consequently, the scientifically correct reporting is IC₅₀ > 1000 µg/mL. The extrapolated value of approximately 1076.29 µg/mL can only be presented as an estimated value and must not replace the official IC₅₀ reporting.

III.7.2. Corrected DPPH inhibition profile

The corrected DPPH inhibition profile showed two clearly distinct behaviours. Ascorbic acid produced a rapid increase in radical inhibition and exceeded the 50% threshold between 40 and 60 µg/mL. By contrast, the *Cornulaca* extract displayed a slower and incomplete response, reaching only 46.61% inhibition at the highest tested concentration of 1000 µg/mL. A slight non-monotonic variation was observed between 100 and 200 µg/mL for the extract, which may reflect experimental variability, extract-matrix effects, solubility/homogeneity limitations or spectrophotometric interaction. This observation does not invalidate the global trend, but it reinforces the need for cautious interpretation and future dose-response confirmation.

Table 20. Corrected DPPH inhibition percentages calculated from raw absorbance values.

Sample	Concentration (µg/mL)	Mean absorbance	SD absorbance	CV absorbance (%)	Corrected inhibition (%)
Ascorbic acid	0	1.1581	0.0020	0.17	0.00
Ascorbic acid	20	0.7124	0.0091	1.27	38.49
Ascorbic acid	40	0.6274	0.0257	4.10	45.83
Ascorbic acid	60	0.5503	0.0441	8.02	52.48
Ascorbic acid	80	0.4417	0.0211	4.77	61.86
Ascorbic acid	100	0.4263	0.0048	1.12	63.19
<i>Cornulaca</i> extract	0	1.1581	0.0020	0.17	0.00

Cornulaca extract	100	0.9855	0.0156	1.58	14.90
Cornulaca extract	200	1.0187	0.0428	4.20	12.04
Cornulaca extract	400	0.8904	0.0097	1.09	23.12
Cornulaca extract	600	0.7883	0.0162	2.06	31.93
Cornulaca extract	800	0.7214	0.0187	2.60	37.71
Cornulaca extract	1000	0.6184	0.0158	2.55	46.61

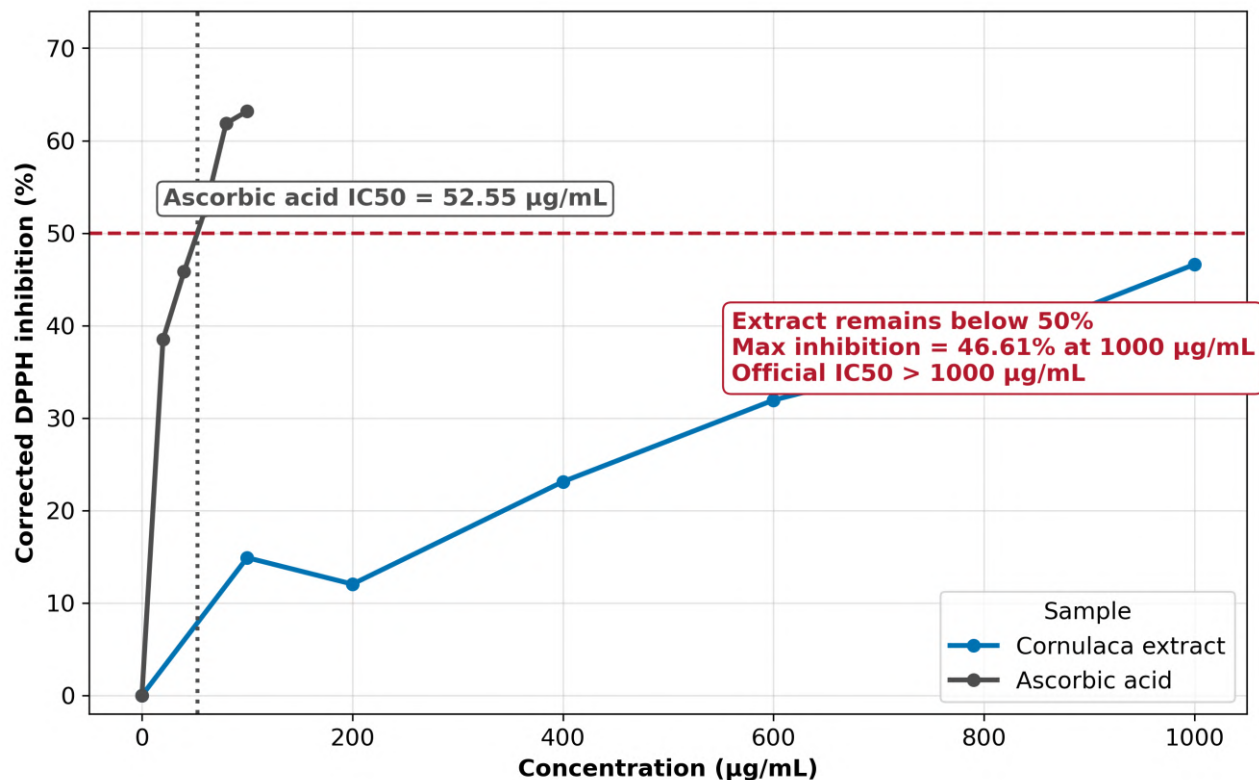


Figure 28. DPPH dose-response curve showing that *C. monacantha* extract did not reach 50% inhibition within the tested concentration range.

III.7.3. IC50 decision and scientific reporting

IC50 values were interpreted according to a strict analytical rule: interpolation is acceptable only when the response curve experimentally crosses the 50% inhibition threshold within the tested concentration range. Ascorbic acid fulfilled this condition and its IC50 was calculated by linear interpolation between 40 µg/mL and 60 µg/mL, giving 52.55 µg/mL. In contrast, the extract did not reach 50% inhibition; therefore, its IC50 cannot be considered experimentally determined. The correct reporting is IC50 > 1000 µg/mL. The extrapolated estimate of 1076.29 µg/mL was obtained from the last two experimental points and is useful only as a descriptive indication of the curve trajectory.

Table 21. DPPH IC₅₀ decision table distinguishing true interpolation from extrapolated estimation.

Sample	Highest tested concentration (µg/mL)	Maximum inhibition (%)	50% reached?	Official IC ₅₀ reporting	Scientific decision
Ascorbic acid	100	63.19	Yes	52.55 µg/mL	True IC ₅₀ by interpolation
Cornulaca extract	1000	46.61	No	>1000 µg/mL	IC ₅₀ not reached; extrapolated value only

Table 22. DPPH comparison with ascorbic acid.

Sample	Official IC ₅₀	Estimated value	Maximum inhibition (%)	Interpretation
Ascorbic acid	52.55 µg/mL	Not needed	63.19	Reference antioxidant; 50% inhibition reached.
Cornulaca extract	>1000 µg/mL	≈1076.29 µg/mL (estimated only)	46.61	Weak DPPH response; 50% inhibition not reached.

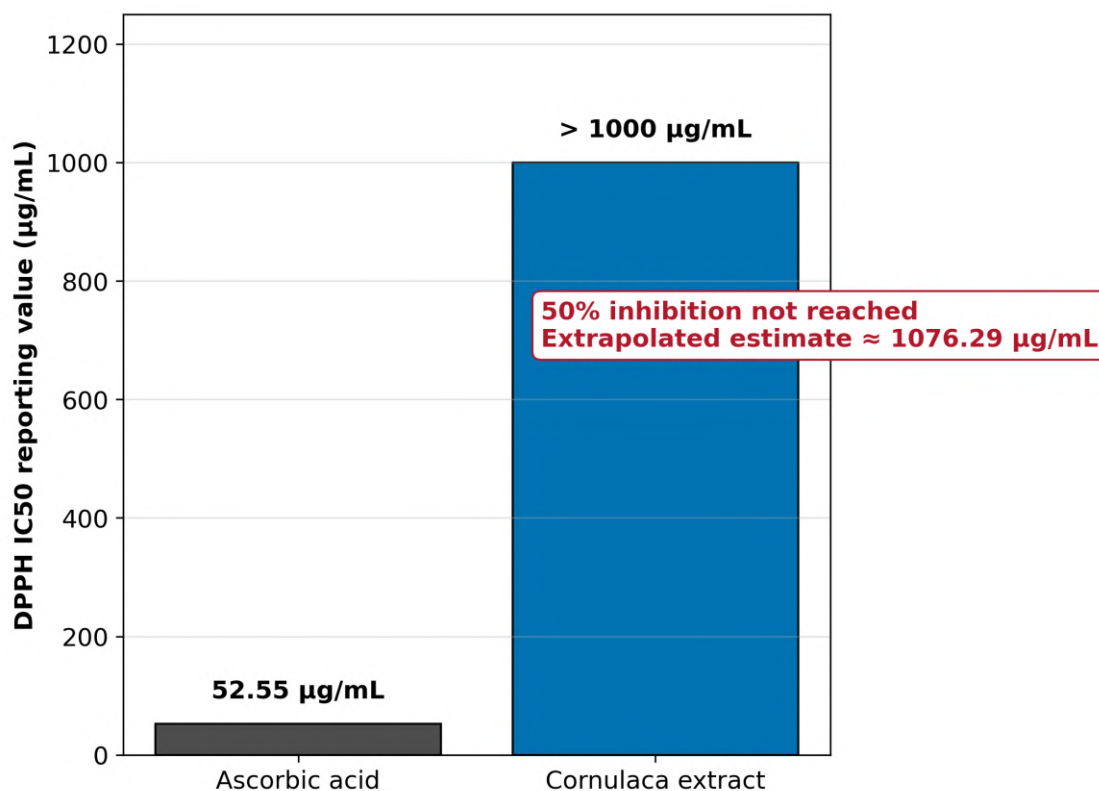


Figure 29. DPPH IC₅₀ comparison; the extract must be reported as IC₅₀ > 1000 µg/mL because 50% inhibition was not achieved.

III.7.4. Biological and phytochemical interpretation

The weak DPPH response of the extract should be interpreted in relation to the chemistry of the assay and the integrated antioxidant profile of the plant. DPPH is a relatively bulky nitrogen radical and is commonly more responsive to antioxidant molecules with appropriate steric accessibility, solubility in the reaction medium and suitable electron/hydrogen donation kinetics. Plant extracts rich in polar phenolics or complex glycosylated/flavonoid-like constituents may show different reactivity in DPPH compared with ABTS or reducing-capacity assays. Thus, the present DPPH result does not negate the antioxidant potential previously suggested by the ABTS response and total antioxidant capacity, but rather indicates an assay-dependent antioxidant behaviour. This is consistent with methodological reviews emphasizing that antioxidant capacity is multidimensional and should be evaluated using complementary assays rather than a single radical system (Huang et al., 2005; Prior et al., 2005; Apak et al., 2016; Gulcin, 2020).

From a results-and-discussion perspective, the most defensible conclusion is that the hydroethanolic extract of *C. monacantha* exhibited a weak-to-moderate DPPH radical scavenging response under the tested conditions. The response increased at higher concentrations but did not reach the conventional 50% inhibition threshold. Accordingly, any sentence stating “IC₅₀ = 1076.29 µg/mL” should be avoided unless the term “extrapolated estimate” is clearly attached to it. The official result remains IC₅₀ > 1000 µg/mL.

The hydroethanolic extract of *C. monacantha* showed a progressive but incomplete DPPH radical scavenging response, reaching 46.61% inhibition at 1000 µg/mL. Since 50% inhibition was not achieved within the experimental range, the IC₅₀ of the extract should be reported as >1000 µg/mL. The extrapolated value of approximately 1076.29 µg/mL is only an estimated value and should not be treated as a true experimentally determined IC₅₀. Under the same conditions, ascorbic acid showed a true interpolated IC₅₀ of 52.55 µg/mL, confirming its markedly stronger DPPH scavenging activity.

III.8. Integrated Antioxidant and Phytochemical Profile

The integrated interpretation of antioxidant and phytochemical results provides a more informative representation of extract performance than the isolated reading of individual assays. Antioxidant capacity is not a single physicochemical property; rather, it emerges from multiple reaction pathways, including single-electron transfer, hydrogen-atom transfer, proton-coupled electron transfer, radical accessibility, metal reduction and matrix-dependent kinetic effects. Consequently, concordance among phytochemical indices and antioxidant assays should be examined together, while divergence among tests must be interpreted as mechanistically informative rather than as an analytical contradiction (Blois, 1958; Miller et al., 1993; Brand-Williams et al., 1995; Re et al., 1999; Huang et al., 2005; Prior et al., 2005; Apak et al., 2016; Gülçin, 2020).

In the present study, the hydroethanolic extract of *C. monacantha* combined a strong qualitative phytochemical signature with moderate quantitative phenolic, flavonoid and reducing-capacity indices. The phytochemical screening indicated strong reactions for polyphenols, flavonoids and terpenoids and a moderate reaction for saponins. Quantitatively, total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) were 21.79 ± 0.50 mg GAE/g extract, 8.42 ± 0.27 mg RE/g extract and 17.43 ± 0.42 mg AAE/g extract, respectively. Radical-scavenging behaviour was assay-dependent: the ABTS IC₅₀ was 185.93 µg/mL, whereas the extract did not reach 50% DPPH inhibition within the tested range and was therefore correctly reported as IC₅₀ > 1000 µg/mL. The DPPH value of approximately 1076.29 µg/mL is retained only as an extrapolated estimate.

Table 23. Integrated bioactive profile of the hydroethanolic extract.

Dimension	Result	Unit	Analytical meaning	Integrated interpretation	Main caution
Qualitative screening	Polyphenols +++; flavonoids +++; terpenoids +++; saponins ++	Ordinal score	Preliminary detection of broad secondary-metabolite classes	Strong qualitative phytochemical profile	Visual reactions are not concentrations or structural identification
TPC	21.79 ± 0.50	mg GAE/g extract	Folin-Ciocalteu reducing/phenolic index	Moderate phenolic richness	Method is not specific to individual phenolics
TFC	8.42 ± 0.27	mg RE/g extract	AlCl ₃ -reactive flavonoid index calibrated with rutin	Moderate flavonoid index	Must be expressed as RE, not QE
TAC	17.43 ± 0.42	mg AAE/g extract	Phosphomolybdenum reducing capacity	Moderate global reducing response	Calibration R ² = 0.9696; improvement is desirable
ABTS	IC ₅₀ = 185.93	µg/mL	ABTS radical-cation scavenging	Favourable response relative to DPPH	Experimental control absorbance differed from the classical target
DPPH	IC ₅₀ > 1000; estimate $\approx$ 1076.29	µg/mL	DPPH radical scavenging	Weak response within the tested range	The estimated IC ₅₀ is extrapolated, not experimentally reached

Table 23 demonstrates that the apparently favourable qualitative screening profile was accompanied by measurable but not exceptionally high quantitative indices. This distinction is important because qualitative color or froth intensity can indicate the presence of reactive chemical classes but cannot predict their concentrations, molecular identities or biological availability. The quantitative assays therefore refine, rather than simply confirm, the qualitative observations. In particular, the moderate TPC and TFC values are compatible with a chemically diverse extract whose antioxidant response may arise from combined contributions of phenolics, flavonoids, terpenoids, saponin-associated constituents and other reducing compounds.

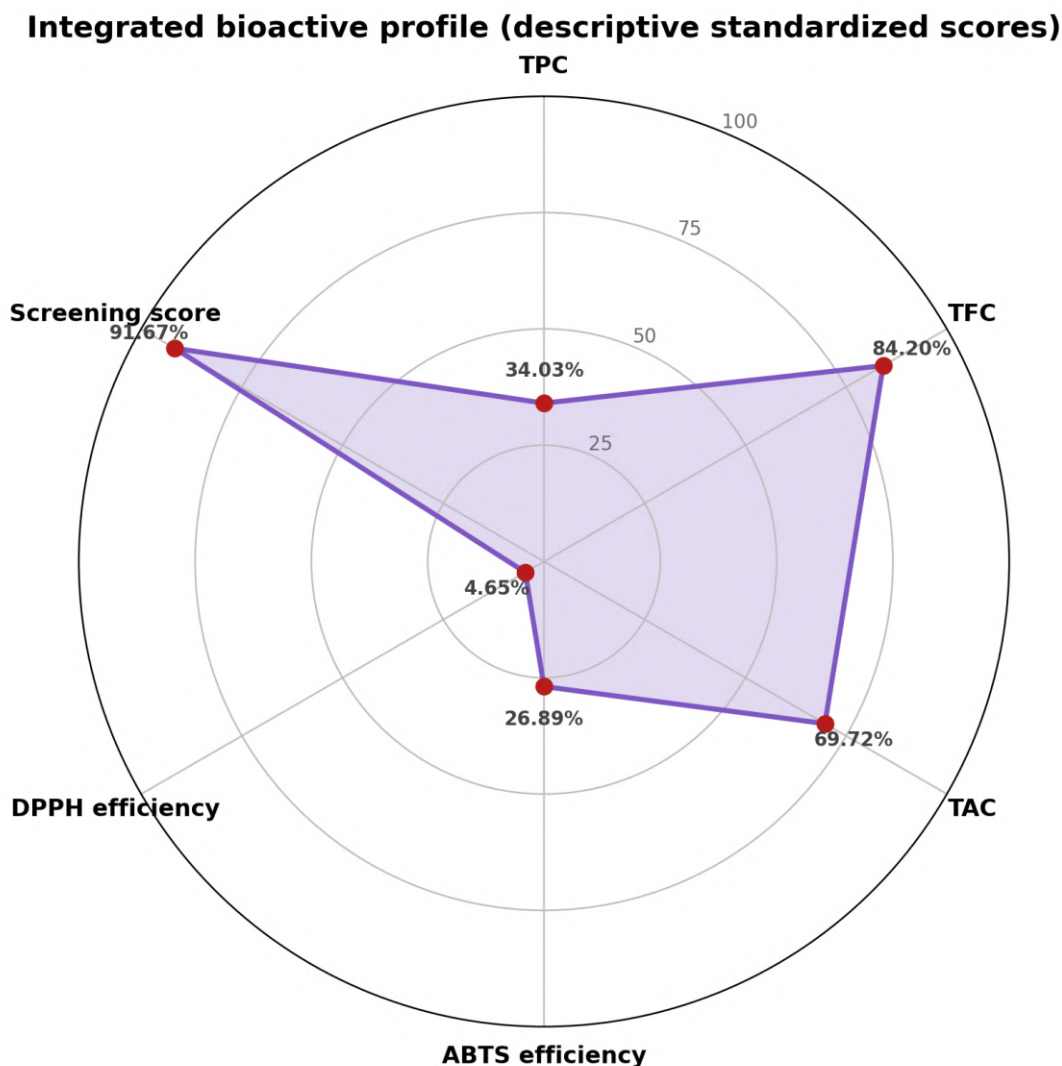
Table 24. Synthetic indices derived from the integrated dataset.

Index	Formula	Value	Interpretive meaning	Critical caution
Phenolic Contribution Index (PCI)	TPC/TAC	1.25	TPC is approximately 1.25 times the TAC index	GAE and AAE are different reference systems; not a stoichiometric contribution
Flavonoid Contribution Ratio (FCR)	TFC/TPC	0.386 (38.6%)	Relative position of the flavonoid index within the phenolic index	RE and GAE are not interchangeable; not a molecular percentage
ABTS Antioxidant Efficiency Index	1/IC ₅₀ ABTS	0.00538 mL/μg	Inverse IC ₅₀ descriptor; larger values indicate lower IC ₅₀	Study-specific descriptive index, not a universal validated scale
DPPH Antioxidant Efficiency Index	1/extrapolated IC ₅₀ DPPH	0.000929 mL/μg	Approximate inverse response for comparison	Derived from extrapolation and therefore secondary
ABTS/DPPH responsiveness ratio	IC ₅₀ DPPH(est.)/IC ₅₀ ABTS	5.79	The extract responded more strongly in ABTS than in DPPH	Uses extrapolated DPPH IC ₅₀ ; does not quantify antioxidant molecules
ABTS extract/reference ratio	185.93/41.62	4.47	Extract was 4.47-fold less active than ascorbic acid in ABTS	Specific to the adopted assay conditions
DPPH extract/reference minimum ratio	1000/52.55	>19.03	Extract was at least 19-fold less active than ascorbic acid in DPPH	A minimum ratio because extract IC ₅₀ exceeded 1000 μg/mL

The synthetic indices summarize relationships among the assays but should not be confused with validated universal biomarkers. The PCI of 1.25 indicates numerical proximity between the phenolic and phosphomolybdenum indices, consistent with a possible contribution of redox-active phenolics to global reducing capacity. Nevertheless, the two values were generated against different standards and by different chemical reactions. Similarly, the FCR of 0.386 indicates that the rutin-equivalent flavonoid index corresponds numerically to approximately 38.6% of the gallic-acid-equivalent phenolic index; it does not establish that flavonoids constitute 38.6% of the extract phenolics on a mass or molar basis.

The contrast between the ABTS and DPPH indices is the most informative component of the integrated analysis. The ABTS/DPPH responsiveness ratio was approximately 5.79 when the extrapolated

DPPH IC₅₀ was used. This pattern is consistent with the known non-equivalence of antioxidant assays. ABTS•⁺ can react with a broad spectrum of hydrophilic and lipophilic electron- or hydrogen-donating compounds, whereas DPPH reactivity can be constrained by solvent compatibility, steric accessibility, reaction kinetics and the chemical structure of antioxidant constituents (Re et al., 1999; Arts et al., 2004; Huang et al., 2005; Prior et al., 2005; Rumpf et al., 2023; Gülçin, 2023). Therefore, a strong ABTS response combined with a weak DPPH response is scientifically plausible and supports an assay-dependent antioxidant phenotype.

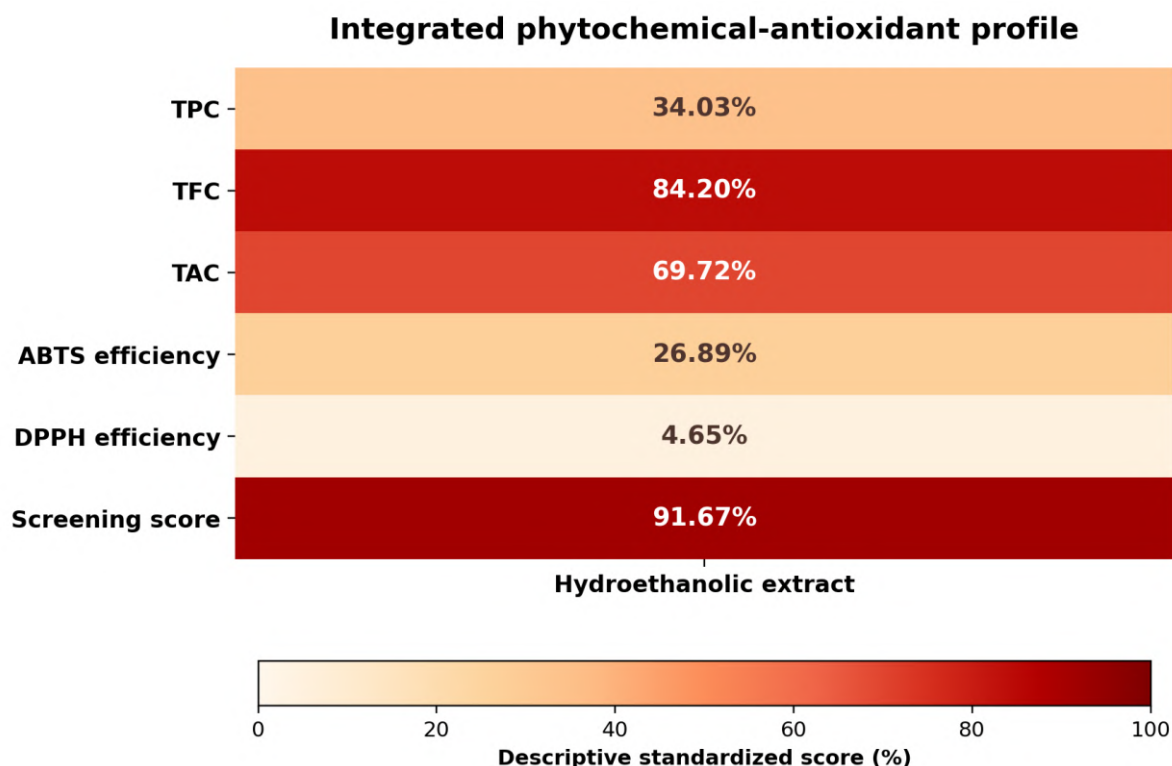


Standardization is used only for graphical integration; the underlying assays have different units and reference standards.

Figure 30. Radar representation of the global bioactive profile. TPC, TFC, TAC, ABTS efficiency, DPPH efficiency and qualitative screening were transformed into descriptive standardized scores solely to permit joint visualization. The plotted percentages must not be interpreted as directly comparable concentrations or biological effect sizes.

Figure 29 emphasizes three dominant features: a high qualitative screening score, a comparatively high scaled TFC signal and a moderate scaled TAC signal. In contrast, DPPH efficiency was markedly

lower than ABTS efficiency. The radar geometry should be treated as a visualization framework rather than as a multivariate statistical analysis. Because the six axes originate from different analytical principles and units, the polygon does not represent an absolute bioactivity score. Its value lies in making the internal pattern of strengths and weaknesses immediately visible.



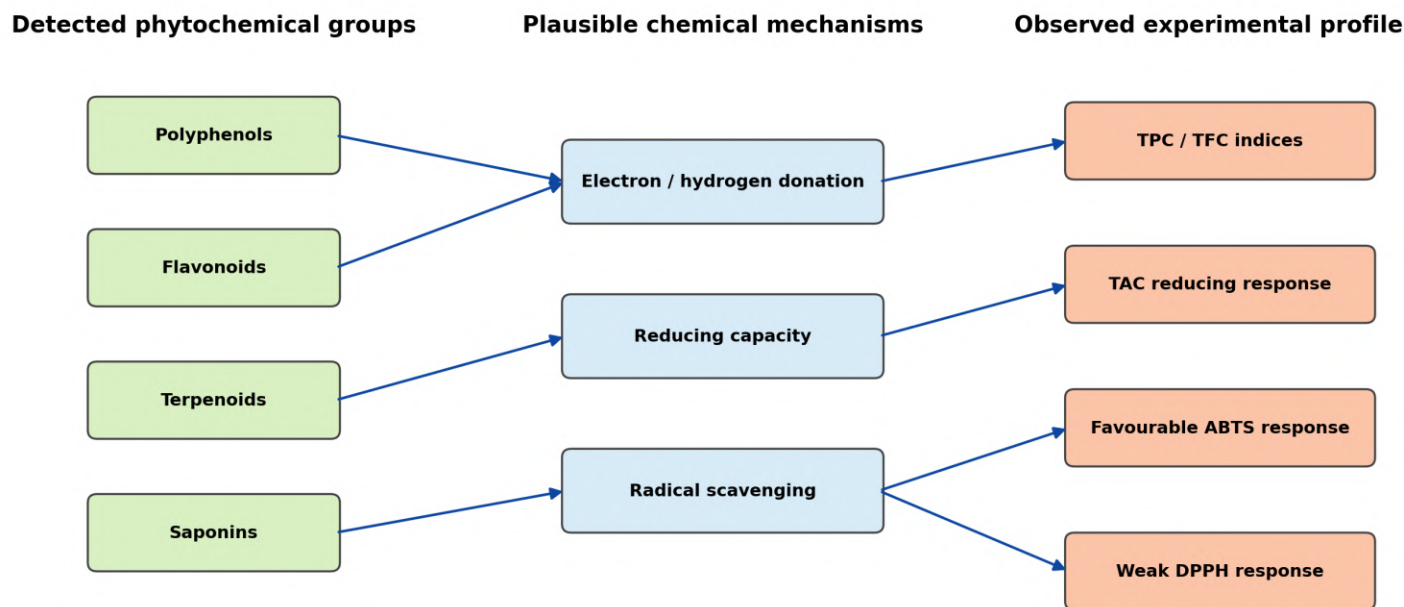
Scores are visualization aids and are not directly comparable concentrations or effect sizes.

Figure 31. Heatmap of the integrated phytochemical-antioxidant profile based on descriptive standardized scores. Darker cells indicate higher positions relative to the predefined visualization references; they do not indicate directly comparable chemical concentrations.

The heatmap reproduces the same pattern in a more compact form. The screening score was the highest standardized descriptor (91.67%), followed by TFC (84.20%) and TAC (69.72%). TPC occupied a lower relative position (34.03%), while ABTS and DPPH efficiency scores were 26.89% and 4.65%, respectively. These percentages are conditional on the selected scaling references and are not intrinsic properties of the extract. Accordingly, their appropriate use is descriptive communication, not inferential ranking.

Table 25. Strengths, weaknesses and interpretive boundaries of the extract.

Aspect	Classification	Evidence	Scientific implication	Recommended interpretation
Qualitative phytochemical profile	Strength	Polyphenols +++; flavonoids +++; terpenoids +++; saponins ++	Supports broad secondary-metabolite diversity	Use as preliminary chemical evidence
Analytical repeatability	Strength	CV = 2.31-3.20% for TPC/TFC/TAC	Good within-assay precision of triplicate measurements	Supports reliability of reported means
ABTS response	Relative strength	IC ₅₀ = 185.93 µg/mL	Relevant radical-cation scavenging under the adopted conditions	Favourable compared with DPPH, but weaker than ascorbic acid
Quantitative richness	Intermediate	Moderate TPC, TFC and TAC values	Bioactive constituents are present but not exceptionally concentrated	Avoid describing the extract as extremely rich
DPPH response	Weakness	Maximum inhibition 46.61% at 1000 µg/mL	Limited DPPH reactivity in the tested range	Report IC ₅₀ >1000 µg/mL
Analytical standardization	Caution	Different standards and one suboptimal calibration curve	Prevents direct numerical equivalence among assays	Interpret integrated indices descriptively
Molecular attribution	Limitation	No LC-MS/MS profile in the current dataset	Active compounds cannot be assigned definitively	Require chromatographic and spectrometric profiling
Biological extrapolation	Limitation	Cellular and in vivo protection were not tested	Chemical antioxidant potential is not proof of physiological protection	Avoid therapeutic or hepatoprotective claims



Proposed interpretation only: these links are consistent with chemical assays but do not establish cellular or in vivo mechanisms.

Figure 32. Proposed conceptual linkage between the detected phytochemical groups, plausible chemical antioxidant mechanisms and the observed experimental profile. The diagram is hypothesis-generating and does not demonstrate cellular or in vivo mechanisms.

The conceptual model in Figure 32 is consistent with established redox chemistry. Phenolic and flavonoid structures may donate electrons or hydrogen atoms and stabilize the resulting radicals through resonance; selected compounds may also participate in transition-metal chelation. Terpenoids can contribute through radical quenching and membrane-associated antioxidant effects, whereas saponins may indirectly modify extract solubility, interfacial behaviour and the accessibility of co-occurring metabolites. However, the present qualitative tests do not identify specific molecules or prove their individual contribution. This distinction is especially relevant for *C. monacantha* because targeted phytochemical investigations have isolated isoflavones, phenolic acids and feruloyltyramine derivatives with antioxidant activity, demonstrating that the species contains structurally diverse redox-active compounds (Mhiri et al., 2020). More recent chemical investigations have further expanded its specialized-metabolite profile, including the isolation of cornulacin and LC-MS/MS-supported profiling in biological models (Badawy et al., 2024; Eltamany et al., 2026).

The current results also agree conceptually with recent work showing that extraction conditions profoundly influence the recovery of phenolics and the resulting antioxidant response. Ultrasound-assisted optimization of *C.monacantha* yielded higher TPC values than the present macerated hydroethanolic extract and demonstrated assay-specific antioxidant behaviour across DPPH, ABTS, CUPRAC and reducing-power systems (Lemmadi et al., 2025). These findings reinforce the interpretation that the present integrated profile reflects both the intrinsic chemistry of the collected material and the operational selectivity of the extraction and assay conditions.

Taken together, the hydroethanolic extract exhibited a coherent but moderate bioactive profile. The strong qualitative reactions indicate broad phytochemical diversity, while the TPC, TFC and TAC measurements establish measurable quantitative richness and reducing capacity. The comparatively favourable ABTS response and weak DPPH response show that antioxidant behaviour cannot be reduced to a single potency value. Instead, the extract appears to contain constituents that react efficiently with the ABTS radical cation but less effectively, or more slowly, with DPPH under the adopted conditions. This assay dependence is a central result of the study and justifies the use of complementary antioxidant methods.

The synthetic indices add communicative value but remain secondary to the original measurements. PCI and FCR should be interpreted as internal descriptive relationships, whereas AEI_ABTS and the ABTS/DPPH ratio summarize inverse IC₅₀ behaviour. None of these indices substitutes for chromatographic identification, absolute quantification of individual metabolites, kinetic characterization or biological validation. Accordingly, the present integrated profile supports the antioxidant potential of *C. monacantha* and identifies specific analytical strengths, but it does not establish cellular antioxidant protection, bioavailability, safety or therapeutic efficacy.

III.9. Comparison with Literature

III.9.1. Rationale and scope of the comparison

The present subsection positions the hydroethanolic extract of *C. monacantha* in relation to the available species-specific literature. The comparison is intentionally descriptive rather than inferential because the published studies are heterogeneous with respect to geographic origin, plant organ, harvest period, post-harvest processing, extraction solvent, extraction technology, solid-to-liquid ratio, calibration standard, analytical protocol, reference antioxidant and reporting basis. Such heterogeneity is particularly consequential in phytochemical research, where solvent polarity and extraction intensity can alter both extraction yield and the spectrum of recovered metabolites (Dai and Mumper, 2010; Khoddami et al., 2013; Azwanida, 2015; Abubakar and Haque, 2020).

Accordingly, numerical values were harmonized only when conversion was dimensionally valid. Values expressed as micrograms of equivalent per milligram of dry extract were converted numerically to milligrams of equivalent per gram of dry extract because $1 \mu\text{g}/\text{mg} = 1 \text{mg}/\text{g}$. In contrast, values expressed against different reference standards (e.g., quercetin equivalents versus rutin equivalents) were retained in their original units, since those standards are not analytically interchangeable. Likewise, ABTS, DPPH and phosphomolybdenum results were not collapsed into a common scale because they probe distinct reaction systems and are influenced by different kinetic, steric and medium effects (Huang et al., 2005; Prior et al., 2005; Apak et al., 2016; Gülçin, 2020).

III.9.2. Comparison of total phenolic content

The total phenolic content (TPC) of the present hydroethanolic extract was $21.79 \pm 0.50 \text{ mg GAE/g}$ extract. This value was lower than the values reported for several *C. monacantha* preparations, including 31.33 mg GAE/g for an aqueous extract from the El-Oued region, $37.27 \pm 0.68 \text{ mg GAE/g}$ dry extract after optimized ultrasound-assisted ethanolic extraction, 64.03 mg GAE/g dry extract for a methanolic extract from the Algerian Sahara, and $29.91 \pm 1.61 \text{ mg GAE/g}$ extract for a recent Egyptian methanolic extract. However, the apparent ranking should not be interpreted as evidence of intrinsic geographic inferiority or superiority. The optimized ultrasound-assisted protocol of Lemmadi et al. used a Box-Behnken design and was specifically developed to maximize phenolic recovery, whereas the present work used conventional 24-h maceration in ethanol/water (50:50, v/v). The higher value reported for the methanolic extract is also consistent with the capacity of methanol to recover a different spectrum of moderately polar phenolics.

Reference	Origin	Plant part	Extraction	Reported / harmonized unit	Value	Comparison	Main limitation
Present study	Bennacer, SE Algeria	Aerial parts	EtOH/H ₂ O 50:50; maceration	mg GAE/g extract	21.79	Reference value	Same species; present protocol
Ali Boutlelis et al.	El-Oued, Algeria	Aerial parts	Aqueous extract	mg GAE/g extract	31.33	Higher	Different solvent and extraction context
Lemmadi et al. (2025)	Algerian Sahara	Aerial parts	Optimized ethanolic UAE	$\mu\text{g GAE}/\text{mg DE} = \text{mg GAE}/\text{g DE}$	37.27	Higher	Optimized extraction; dry-extract basis

Boussadia et al. (2024)	Algerian Sahara	Aerial parts	Methanolic extract	$\mu\text{g GAE/mg DE} = \text{mg GAE/g DE}$	64.03	Much higher	Methanol; different origin and workflow
Eltamany et al. (2026)	Egypt	Aerial parts	Methanolic crude extract	$\mu\text{g GAE/mg extract} = \text{mg GAE/g extract}$	29.91	Higher	Different origin; LC-MS-supported study context

III.9.3. Comparison of total flavonoid content

The total flavonoid content (TFC) of the present extract was 8.42 ± 0.27 mg RE/g extract. The use of rutin as the calibration standard is analytically decisive: the result must be expressed as rutin equivalents (RE), not quercetin equivalents (QE). The recent Egyptian study likewise used rutin and reported 6.30 ± 0.37 mg RE/g extract, making this comparison more methodologically coherent than comparison with the 7.21 ± 0.31 mg QE/g dry extract reported by Lemmadi et al. Because rutin and quercetin differ in molar absorptivity and complex formation in the aluminium chloride assay, RE and QE values are not directly interchangeable. The numerically higher present value relative to the recent rutin-based result may reflect extraction solvent composition, geographic provenance, phenological state or differences in analytical conditions rather than a definitive difference in flavonoid abundance.

Table 27. Literature comparison of total flavonoid content and flavonoid-related evidence.

Reference	Standard	Unit / evidence	Value	Comparability	Interpretive note
Present study	Rutin	mg RE/g extract	8.42	Direct reference	Correct unit because rutin was used
Lemmadi et al. (2025)	Quercetin	mg QE/g dry extract	7.21	Partial only	Different standard and extraction basis
Eltamany et al. (2026)	Rutin	mg RE/g extract	6.30	Relatively comparable	Same equivalent standard; different origin/extraction
Kandil & Grace (2001)	Not applicable	Isolated compounds	—	Qualitative support	Luteolin glycosides and gallotannins identified
Mhiri et al. (2020)	Not applicable	Isolated compounds	—	Qualitative support	New isoflavones and related phenolics identified
Badawy et al. (2024)	Not applicable	Isolated compounds	—	Qualitative support	Cornulacin and additional phenylpropanoid amides isolated

III.9.4. Comparison of antioxidant assays

The present ABTS IC₅₀ was 185.93 $\mu\text{g/mL}$, compared with 41.62 $\mu\text{g/mL}$ for ascorbic acid under the same experimental conditions. Species-specific ABTS data reported with the same metric, crude-extract type and reference antioxidant remain scarce. Published work on isolated constituents demonstrates antioxidant potential, but isolated molecules cannot be ranked directly against a crude hydroethanolic extract. For DPPH, the present extract did not attain 50% inhibition at the highest tested concentration; its scientifically valid result is therefore IC₅₀ > 1000 $\mu\text{g/mL}$. The extrapolated value of approximately 1076.29 $\mu\text{g/mL}$ is retained only as a secondary estimate. Boussadia et al. reported an IC₅₀ of approximately 0.62 mg/mL (620 $\mu\text{g/mL}$) for a methanolic extract, indicating stronger DPPH scavenging than the present extract, but the different solvent, plant provenance and possibly assay configuration limit direct interpretation. A recent Egyptian methanolic extract showed a DPPH IC₅₀ of 346.4 ± 8 $\mu\text{g/mL}$ against Trolox, further illustrating how extraction chemistry and reference selection materially alter the observed potency.

Table 28. Literature comparison of antioxidant assays.

Assay	Reference	Sample type	Metric	Reported value	Reference antioxidant	Comparability
ABTS	Present study	Hydroethanolic crude extract	IC50	185.93 µg/mL	Ascorbic acid	Reference result
ABTS	Mhiri et al. (2020)	Isolated compounds	Compound-level antioxidant assays	Different metric	—	Mechanistic support; not directly comparable
DPPH	Present study	Hydroethanolic crude extract	IC50	>1000 µg/mL	Ascorbic acid	50% inhibition not reached
DPPH	Boussadia et al. (2024)	Methanolic crude extract	IC50	620 µg/mL	Ascorbic acid	Partial comparison; different solvent
DPPH	Eltamany et al. (2026)	Methanolic crude extract	IC50	346.4 ± 8 µg/mL	Trolox	Limited comparison; different reference
TAC	Present study	Hydroethanolic crude extract	Phosphomolybdenum	17.43 mg AAE/g	Ascorbic acid	Reference result
TAC	Mhiri et al. (2020)	Isolated compounds	Phosphomolybdenum-like capacity	Vitamin E equivalents	Vitamin E	Different sample type and unit

III.9.5. Graphical benchmarking

Figure 33 summarizes the species-specific TPC and TFC values after dimensionally valid unit harmonization. The TPC panel shows that the present extract occupies the lower end of the published range, whereas the TFC panel shows a value that is numerically higher than the two recent literature values. The TFC panel must nevertheless be read with the explicit caveat that one literature value is expressed in QE and the others in RE. Figure 34 compares IC50 values; lower values indicate stronger activity. The DPPH bar for the present extract represents the tested lower bound (>1000 µg/mL), not an experimentally measured IC50.

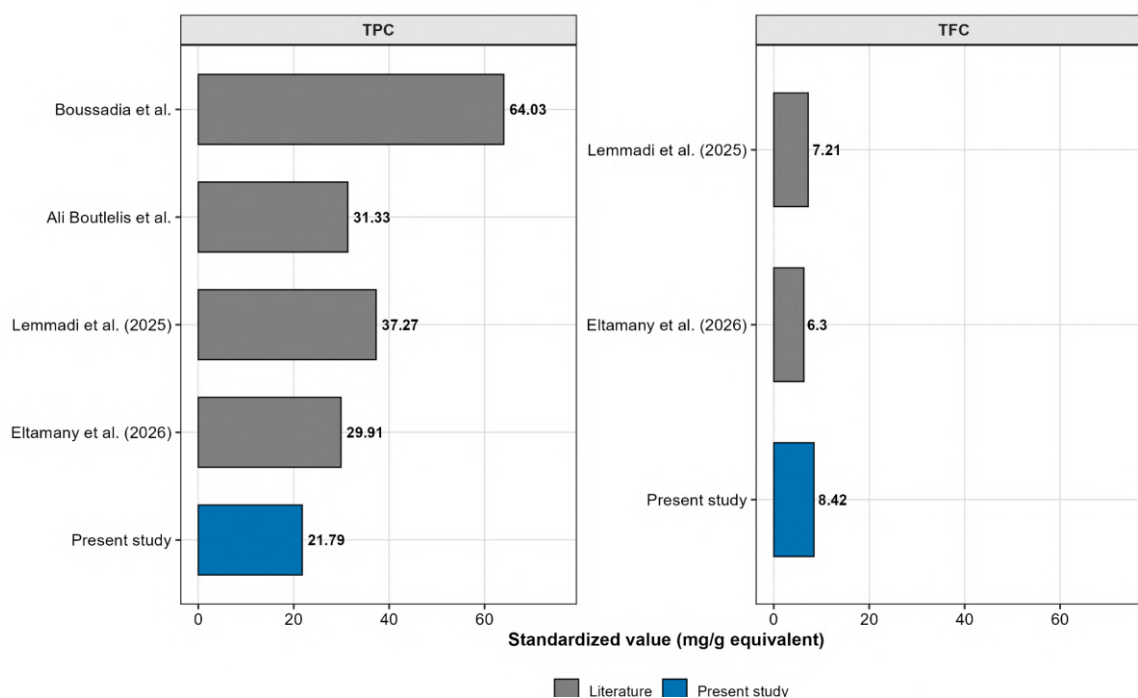


Figure 33. Benchmark plot for total phenolic content (TPC) and total flavonoid content (TFC) of *C. monacantha*. The comparison is descriptive; TFC values based on rutin and quercetin standards are not directly interchangeable.

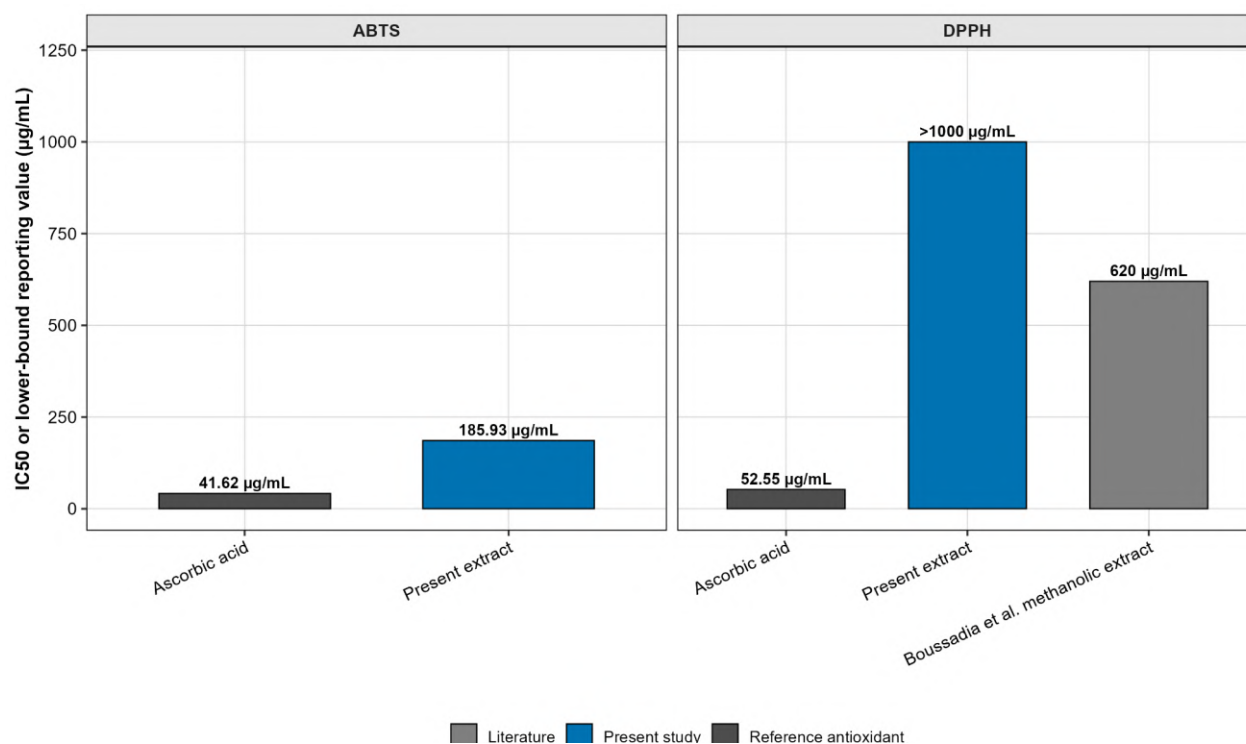


Figure 34. Comparative antioxidant activity plot. Lower IC₅₀ values indicate stronger radical-scavenging activity. The present DPPH extract value is reported as >1000 µg/mL because 50% inhibition was not achieved within the tested range.

III.9.6. Methodological interpretation and limits of cross-study comparison

The lower TPC of the present extract should therefore be interpreted as a property of the specific plant–solvent–method system examined, rather than as an immutable characteristic of the species. Extraction selectivity is governed by solvent polarity, water content, temperature, time, particle size, agitation and solid-to-liquid ratio. Ultrasound-assisted extraction can intensify mass transfer and cell-wall disruption, whereas methanol may recover compounds that are less efficiently extracted by a 50% ethanol system. Conversely, hydroethanolic extraction is more compatible with future food or botanical-ingredient applications and may be preferable from a translational perspective despite a lower spectrophotometric TPC.

The comparison also highlights the limitations of global spectrophotometric indices. The Folin-Ciocalteu reagent measures overall reducing capacity and is not specific to phenolics; the aluminium chloride method is structurally selective and its response varies among flavonoid subclasses; and antioxidant IC₅₀ values depend on radical concentration, incubation time, solvent, pH and curve-fitting strategy. Consequently, chromatographic profiling by HPLC-DAD and LC-MS/MS is required to determine whether differences in global indices correspond to differences in individual metabolites. This need is reinforced by the identification in *C. monacantha* of luteolin glycosides, gallotannins, novel isoflavones, vanillic acid, feruloyltyramines and cornulacin (Kandil & Grace, 2001; Mhiri et al., 2020; Badawy et al., 2024).

No inferential statistical test was applied against literature values. Such testing would be invalid without raw replicate-level data generated under harmonized protocols. The present comparison should therefore be read as a contextual benchmark that identifies the plausible influence of extraction and analytical variables, not as a formal meta-analysis.

III.9.7. Overall synthesis

Taken together, the literature comparison positions the present hydroethanolic extract as a preparation with moderate phenolic richness, a flavonoid index comparable to or slightly higher than recent species-specific estimates, a relevant ABTS response and comparatively weak DPPH scavenging. The available evidence supports genuine phytochemical and antioxidant potential in *C. monacantha*, but also demonstrates substantial method dependence. Direct ranking across studies should therefore remain subordinate to explicit consideration of plant origin, extraction technology, calibration standard, assay chemistry and reporting unit.

III.10. Biological Relevance and Hepatoprotective Perspective

III.10.1. Evidentiary boundary

The present results establish only cell-free chemical reactivity: qualitative phytochemical responses, a robust TPC estimate, provisional TFC and TAC estimates, an interpolated ABTS IC₅₀ and a DPPH IC₅₀ above 1000 µg/mL. They do not demonstrate hepatocyte protection, modulation of intracellular oxidative stress, bioavailability, oral safety or improvement of liver function.

The traditional hepatic indication of *C. monacantha* and the known redox biology of liver injury provide a rationale for selecting future hepatic models, but they cannot be used to infer mechanism or efficacy from the present assays. The appropriate position is therefore that the extract is a candidate for further investigation, not a hepatoprotective preparation.

III.10.2. Evidence matrix

Table 29 separates evidence directly generated by the present study from outcomes that remain hypothetical or unevaluated. This matrix prevents translation of chemical antioxidant activity into unsupported therapeutic or safety claims.

Table 29. Evidence matrix defining what is demonstrated and what remains unproven.

Domain	Present evidence	Evidence status	Scientific interpretation	Conclusion
Qualitative phytochemical richness	Polyphenols +++; flavonoids +++; terpenoids +++; saponins ++	Demonstrated	Supports the presence of major secondary-metabolite families.	Supported
Total phenolic content	21.79 ± 0.50 mg GAE/g extract	Demonstrated	Moderate phenolic index under the adopted assay conditions.	Supported
Total flavonoid content	8.42 ± 0.27 mg RE/g extract	Demonstrated	Moderate rutin-equivalent flavonoid index.	Supported
Total antioxidant capacity	17.43 ± 0.42 mg AAE/g extract	Demonstrated	Moderate phosphomolybdenum reducing capacity.	Supported
Radical-scavenging potential	ABTS IC ₅₀ = 185.93 µg/mL; DPPH IC ₅₀ >1000 µg/mL	Partially supported	Antioxidant potential is assay-dependent and more favorable in ABTS.	Supported with caution
Intracellular antioxidant protection	No hepatocyte oxidative-stress model performed	Not demonstrated	No evidence of reduced intracellular ROS, lipid peroxidation or redox restoration.	Not proven
Direct hepatoprotective activity	No cellular or in vivo liver-injury model performed	Not demonstrated	Cannot be claimed from cell-free chemical assays.	Not proven
Cytotoxicity safety	No HepG2/HepaRG viability assay performed	Not demonstrated	A non-cytotoxic concentration range	Not proven

			has not been established.	
Oral safety	No acute or repeated-dose oral toxicity study performed	Not demonstrated	Required before repeated oral use or supplement development.	Not proven
Bioavailability	No absorption, stability or pharmacokinetic assessment	Not demonstrated	Systemic exposure to active constituents is unknown.	Not proven
Clinical efficacy	No human intervention study	Not demonstrated	No liver-function or therapeutic claim is justified.	Not proven

III.10.3. Biological relevance of the current antioxidant profile

The moderate TPC, TFC and TAC values indicate that the extract contains a chemically active pool of reducing constituents, while the qualitative screening suggests contributions from multiple metabolite classes. The more favorable ABTS response relative to DPPH is consistent with assay-dependent accessibility and reaction mechanisms in a heterogeneous extract. This pattern can justify testing whether non-cytotoxic extract concentrations attenuate intracellular oxidant burden, but it cannot predict the magnitude of protection in hepatocytes.

Phenolic and flavonoid constituents can participate in electron or hydrogen donation, metal chelation and modulation of redox-sensitive signaling; terpenoids and saponins may also contribute through membrane, inflammatory or signaling effects. Nevertheless, global colorimetric indices do not identify the molecules responsible for activity. LC-MS/MS or HPLC-DAD profiling is therefore a high-priority next step, both for mechanistic interpretation and for selection of marker compounds needed to standardize future extract batches.

III.10.4. Required cellular validation

A sequential cellular program should begin with basal cytotoxicity testing in hepatic models. HepG2 cells provide a practical and reproducible screening platform, whereas differentiated HepaRG cells retain a broader range of hepatic metabolic functions and may offer greater relevance for xenobiotic bioactivation. Concentration–viability curves should establish a non-cytotoxic range and, where the response permits, a CC50 with a 95% confidence interval. Protective experiments should then be performed only within the non-cytotoxic concentration range.

Hydrogen peroxide or tert-butyl hydroperoxide can be used to induce oxidative stress in vitro. Carbon tetrachloride is more appropriate as an in vivo hepatotoxicant because its toxicity depends on cytochrome-P450-mediated bioactivation; its use in a cell line requires explicit demonstration of metabolic competence. The experimental design should include untreated controls, vehicle controls, extract-only controls, stressed controls, a positive protective control and several extract concentrations under pre-treatment, co-treatment or post-treatment conditions.

III.10.5. Proposed validation assays and biological endpoints

Protection should be documented across complementary endpoint families. Cell viability and LDH release define cytotoxicity and membrane injury. Intracellular ROS, malondialdehyde or TBARS quantify oxidant burden and lipid peroxidation. GSH, GSSG and the GSH/GSSG ratio characterize redox status, whereas SOD, CAT and GPx activities assess endogenous antioxidant defenses. ALT and AST may be measured, but in cell systems they should be interpreted together with LDH, morphology and apoptosis markers. Mechanistic confirmation may include NRF2 nuclear translocation, HO-1, NQO1, NF- κ B, inflammatory cytokines and apoptosis-related proteins.

Table 30. Prioritized assays proposed for hepatoprotective, safety and product-development validation.

Validation stage	Proposed assay	Biological endpoint	Expected output	Priority	Scientific purpose
Chemical profiling	LC-MS/MS, HPLC-DAD or UPLC-HRMS	Chemical fingerprint and marker compounds	Identity and abundance of candidate metabolites	Very high	Links activity to defined constituents and enables standardization.
Batch standardization	Independent extraction batches	Inter-batch reproducibility	Marker ranges, yield and assay acceptance limits	Very high	Required for reliable biological testing and future formulation.
Cellular safety	HepG2 and/or differentiated HepaRG viability assays	Viability, LDH release and morphology	Non-cytotoxic range and CC50 with IC95%	Very high	Must precede any protection experiment.
Oxidative-stress model	H2O2 or tert-BHP challenge	Cell survival and intracellular ROS	Protection curve and EC50 where estimable	Very high	Directly tests the oxidative-stress protection hypothesis.
Lipid peroxidation	MDA/TBARS and, if possible, 4-HNE	Membrane oxidative damage	Reduction relative to stressed control	High	Lipid peroxidation is central to hepatocellular injury.
Glutathione status	GSH, GSSG and GSH/GSSG ratio	Cellular redox balance	Preservation or restoration of glutathione status	High	GSH is a major hepatic antioxidant defense.
Antioxidant enzymes	SOD, CAT, GPx; optionally GR/GST	Endogenous antioxidant defense	Preservation or normalization of enzyme activities	High	Distinguishes chemical scavenging from cellular defense modulation.
Hepatocellular injury	ALT, AST and LDH; morphology and apoptosis	Cell membrane and death-related injury	Reduction in injury markers without cytotoxicity	High	Provides direct evidence of hepatic-cell protection.
Mechanistic signaling	NRF2 nuclear translocation, HO-1, NQO1; NF- κ B and apoptosis markers	Redox, inflammatory and death pathways	Mechanistic confirmation	Medium to high	Required before proposing a molecular mechanism.
Dose-response modelling	Nonlinear concentration-response analysis	CC50, EC50, maximal effect and IC95%	Quantitative potency and uncertainty	Very high	Supports reproducible comparison and selectivity calculations.

In vivo safety	OECD-aligned acute and repeated-dose studies	Clinical signs, biochemistry, hematology and histology	NOAEL or safety profile	High	Required before repeated oral exposure.
In vivo hepatoprotection	CCl ₄ , paracetamol or ethanol model after cellular validation	ALT/AST, MDA, GSH, enzymes and histopathology	Biochemical and histological evidence	High	Required before claiming hepatoprotective efficacy.
Formulation and stability	Marker-based stability and shelf-life testing	Quality during storage	Stable formulation and specifications	Medium	Required for botanical-ingredient development.

III.10.6. Dose-response and statistical framework for future studies

Future studies should distinguish technical replicates from independent biological experiments. At least three independent experiments are recommended for preliminary cellular validation, with technical wells nested within each experiment. Dose-response curves should be fitted using nonlinear regression when the concentration range spans the relevant response. CC₅₀ should describe cytotoxic potency, whereas EC₅₀ may describe restoration of viability or normalization of a selected stress marker. A selectivity index, CC₅₀/EC₅₀, may be informative only when both parameters are estimable from adequately sampled curves.

For group comparisons, the analytical plan should be selected after examination of residual distribution and variance structure. One-way ANOVA with Tukey adjustment, Welch ANOVA with Games–Howell comparisons, or Kruskal–Wallis with multiplicity-adjusted Dunn tests may be used as appropriate. Factorial designs can evaluate concentration-by-stressor or treatment-by-time interactions, and mixed-effects models can account for independent experimental runs or plates. Results should include biological n, mean and standard deviation, effect sizes, adjusted p-values and 95% confidence intervals.

III.10.7. Translational roadmap

The translational sequence in Figure 35 separates completed chemical evidence from the validation required before formulation or liver-support positioning. Chemical fingerprinting and cellular safety precede efficacy testing; in vivo safety and efficacy precede product-development or clinical claims.

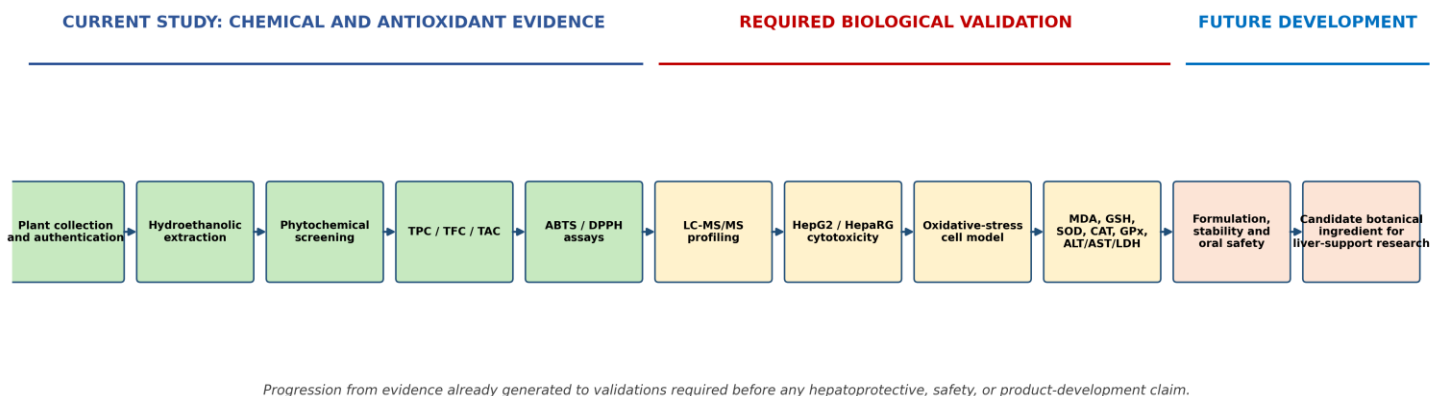
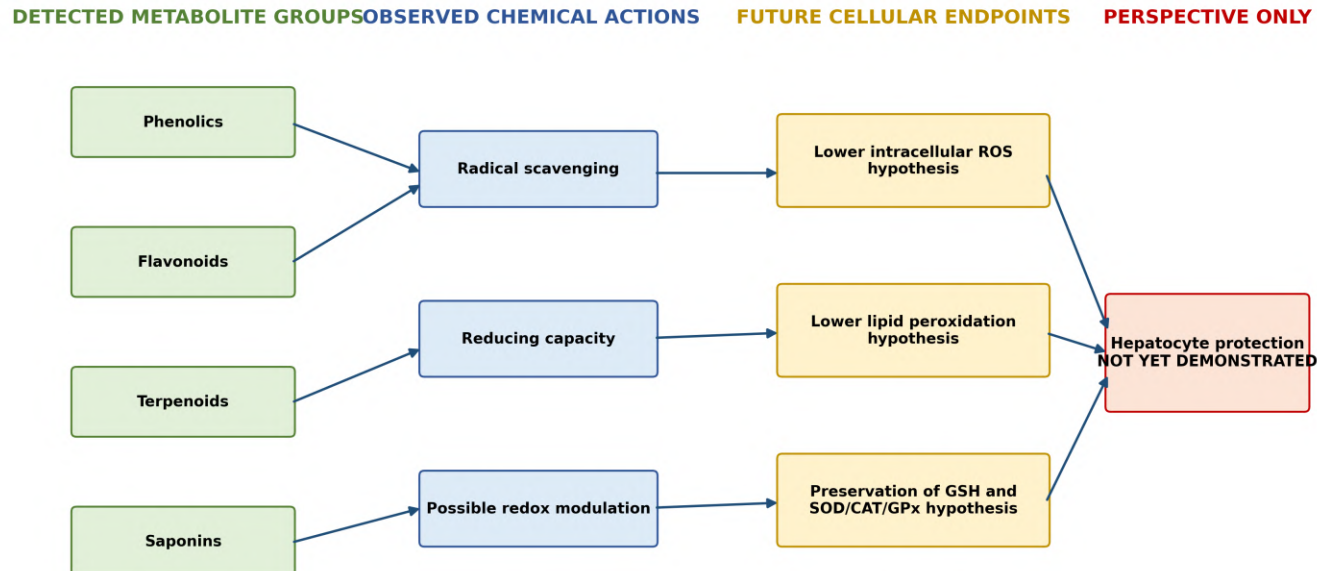


Figure 35. Translational roadmap from extract preparation and cell-free antioxidant assays to chemical standardization, hepatic cellular validation, safety assessment and future botanical-ingredient development.

III.10.8. Proposed mechanism of liver-support potential

Figure 34 presents an explicitly hypothetical framework. The left and central components are supported by the present phytochemical and chemical-assay results. The cellular endpoints and hepatocyte protection shown on the right remain untested and must not be represented as established mechanisms.



Observed steps are restricted to phytochemical and cell-free antioxidant assays; cellular and hepatoprotective steps remain hypotheses requiring experimental validation.

Figure 36. Proposed mechanistic framework linking detected metabolite groups and observed chemical antioxidant actions to future cellular endpoints. Hepatocyte protection has not yet been demonstrated.

III.10.9. Safety, formulation and regulatory perspective

Development as a botanical ingredient would require evidence well beyond the current chemical screening. In the Algerian context, the eventual regulatory category would depend on composition, intended use, dosage form and claims. International FDA, EMA, WHO and OECD documents may guide quality and safety planning, but they do not replace assessment against applicable Algerian food, consumer-protection, public-health and pharmaceutical requirements.

A realistic valorization pathway would begin with a standardized dry extract rather than a finished commercial supplement. The first prototype should specify plant origin, extraction ratio, residual ethanol, moisture, microbial quality and one or more chromatographic markers. Only after calibration revalidation, independent-batch reproducibility, contaminant testing and preliminary safety could a simple oral dosage form for example a marker-standardized capsule or sachet be considered for stability and acceptability studies. No marketing or liver-support claim is justified at the present stage.

III.10.10. Overall conclusion

The study supports a preliminary phytochemical-antioxidant research candidate. It does not support hepatoprotective efficacy, oral safety, nutritional value or immediate supplement development. The next decision gate is analytical revalidation and chemical standardization; cellular and in vivo work should proceed only if those requirements and basic safety criteria are met.

III.11. Limitations and Perspectives

III.11.1. Purpose and interpretive framework

Explicit recognition of methodological limitations is essential for the scientific interpretation of phytochemical and antioxidant studies. The present work established a coherent preliminary profile for the hydroethanolic extract of *C. monacantha*; however, the strength of the conclusions is constrained by the experimental design, the performance of some calibration curves, the absence of chromatographic identification, and the lack of biological and toxicological validation. These limitations do not invalidate the measured results. Instead, they define the level of evidence that can legitimately be claimed and guide the design of the next experiments.

The proposed perspectives were ranked descriptively as high, medium or low priority. This ranking is a planning instrument rather than an inferential analysis. High-priority actions are those required to confirm analytical validity, establish safety, or determine whether progression to the next research stage is justified. Medium-priority actions support optimization, standardization and translation. No p-values or formal statistical comparisons were applied to the priority categories.

III.11.2. Experimental-design and extraction limitations

The study evaluated one hydroethanolic extract prepared from aerial parts under a single maceration condition. Consequently, the findings describe the selected plant-material–solvent–method combination and cannot be generalized to all *C. monacantha* preparations. Solvent polarity, water proportion, temperature, time, particle size, solid-to-liquid ratio and extraction technology can substantially alter both extraction yield and metabolite selectivity. A comparison of aqueous, ethanolic and hydroethanolic systems, together with ultrasound-assisted or other intensified extraction approaches, would clarify whether the current preparation offers the best compromise among chemical yield, antioxidant response, safety, scalability and intended use.

A second design limitation is the absence of clearly demonstrated independent extraction batches. Triplicate spectrophotometric readings quantify analytical repeatability within the available extract, but they do not establish extraction reproducibility. Future work should therefore prepare and analyze multiple independent batches and report inter-batch variation in extraction yield, marker compounds and functional assays.

III.11.3. Analytical and calibration limitations

The gallic-acid calibration displayed excellent linearity, whereas the rutin and ascorbic-acid calibrations produced R^2 values of 0.9595 and 0.9696. Although these curves supported preliminary calculations, R^2 alone is not sufficient to demonstrate method validity, and the lower values indicate that the TFC and TAC calibrations should be repeated. A stronger validation should include a predefined linear range, replicate standards, residual inspection, lack-of-fit assessment where feasible, accuracy or recovery, repeatability, intermediate precision, robustness and fit-for-purpose detection and quantification limits. These principles are consistent with the lifecycle and performance-characteristic approach described in ICH Q2(R2) and Q14.

The ABTS experiment also used an initial control absorbance near 0.322, below the approximately 0.70 value commonly used in the classical radical-cation decolorization procedure. The calculations

remain internally valid because they were based on the actual experimental control, but external comparison is weakened. Future analyses should standardize radical-generation time, initial absorbance, blank correction, incubation time and temperature. For DPPH, the extract did not reach 50% inhibition at 1000 µg/mL. The correct report is therefore IC₅₀ >1000 µg/mL; the estimated 1076.29 µg/mL value should remain explicitly labelled as extrapolated.

III.11.4. Chemical-characterization limitations

The qualitative reactions and global TPC, TFC and TAC indices do not identify the compounds responsible for the observed activity. Colorimetric assays may also respond to structurally diverse reducing substances and cannot substitute for molecular profiling. LC-MS/MS, HPLC-DAD or UPLC-HRMS should therefore be used to establish a reproducible chemical fingerprint, confirm previously reported phenolics and flavonoids of *C. monacantha*, and select quantitative markers for batch standardization.

III.11.5. Biological, toxicological and translational limitations

ABTS, DPPH and phosphomolybdenum are cell-free assays. They do not demonstrate membrane permeability, intracellular antioxidant action, maintenance of glutathione, modulation of antioxidant enzymes or protection of hepatocytes. No HepG2 or HepaRG cytotoxicity study was performed, and no non-cytotoxic concentration range, CC₅₀ or selectivity window is available. Likewise, no oxidative-stress liver-cell model, animal model, oral-toxicity study, bioavailability assessment, formulation study or clinical investigation was conducted.

The absence of these studies is particularly important because botanical-product development requires consistent identity and quality, chemical or biological specifications, stability and an exposure-relevant safety package. Current EMA guidance for herbal medicinal products emphasizes quality specifications and consistency, FDA botanical-drug guidance emphasizes development and control of complex botanical mixtures, and OECD Test Guidelines provide standardized frameworks for repeated-dose oral toxicity. Thus, the present extract can be proposed only as a candidate for further investigation, not as a validated hepatoprotective or orally safe ingredient.

Table 31. Methodological limitations of the present study and recommended corrective actions.

Category	Specific limitation	Consequence for interpretation	Severity	Recommended corrective action
Experimental design	A single hydroethanolic extract and one extraction condition were studied.	The findings cannot be generalized to all solvents, plant organs or extraction technologies.	Medium	Prepare independent extract batches and compare solvents, organs and extraction methods.
Extraction reproducibility	Analytical triplicates were available, but independent extraction batches were not demonstrated.	Technical repeatability does not establish batch-to-batch reproducibility.	High	Produce at least three independent extraction batches and quantify inter-batch variation.
Calibration quality	TFC and TAC calibration curves showed R ² values of	The results are suitable for preliminary	High	Repeat calibration with more levels, replicate standards,

	0.9595 and 0.9696, respectively.	interpretation but not for full analytical validation.		residual analysis and a verified linear range.
Method validation	LOD, LOQ, accuracy, recovery, robustness and measurement uncertainty were not established.	The analytical performance characteristics remain incompletely defined.	High	Validate relevant procedures following fit-for-purpose principles and ICH Q2(R2)/Q14 concepts.
Qualitative screening	The +/+/+++ scores were based on visual color or froth reactions.	Intensity scores cannot be interpreted as concentrations.	Medium	Retain them as descriptive results and complement them with chromatographic or quantitative assays.
Chemical identification	No LC-MS/MS, HPLC-DAD or UPLC-HRMS fingerprint was generated.	The compounds responsible for activity remain unidentified.	High	Establish a chemical fingerprint and quantify selected marker compounds.
ABTS control conditions	The experimental ABTS control absorbance was approximately 0.322, below the classical ~0.70 target.	External comparability with standard ABTS protocols is reduced.	Medium	Standardize radical preparation, starting absorbance, incubation time, blank correction and temperature.
DPPH concentration range	The extract reached only 46.61% inhibition at 1000 µg/mL.	The true IC50 was not experimentally reached and must be reported as >1000 µg/mL.	Medium	Extend the soluble concentration range and repeat concentrations surrounding 50% inhibition.
Antioxidant model	ABTS, DPPH and TAC were cell-free chemical assays.	Chemical radical scavenging does not demonstrate intracellular protection.	High	Introduce hepatic cell-based oxidative-stress models at non-cytotoxic concentrations.
Cytotoxicity	No HepG2 or HepaRG viability evaluation was performed.	A safe cellular concentration range and therapeutic window are unknown.	High	Determine viability, LDH release, CC50 and maximum non-cytotoxic concentration.
Hepatoprotective validation	No cellular or in vivo liver-injury model was performed.	Hepatoprotection remains a hypothesis.	High	Use oxidative-stress liver-cell models, followed by validated in vivo models after safety evaluation.
Oral safety	No acute or repeated-dose oral toxicity assessment was conducted.	Safety for repeated oral exposure cannot be inferred.	High	Conduct OECD-aligned acute and repeated-dose toxicity studies.
Bioavailability	Absorption, metabolism and	The biologically available dose of	Medium	Evaluate stability in gastrointestinal conditions,

	systemic exposure were not investigated.	active metabolites is unknown.		permeability and pharmacokinetics.
Formulation and stability	No formulation, shelf-life or marker-stability study was conducted.	Botanical-ingredient development is premature.	Medium	Develop specifications, stability-indicating methods and a marker-standardized formulation.
Clinical translation	No clinical study was performed.	No health, liver-function or therapeutic claim is justified.	High	Consider clinical work only after analytical, toxicological and efficacy validation.

III.11.6. Prioritized perspectives

The future program should proceed through three sequential stages. The first stage strengthens analytical validity and extract standardization. The second stage establishes cellular safety and tests biological protection under controlled oxidative stress. The third stage evaluates systemic safety, in vivo efficacy, bioavailability, formulation and stability. Progression should be conditional: failure to demonstrate reproducible chemistry, a non-cytotoxic concentration range or a biologically meaningful protective effect should trigger reformulation or re-optimization rather than automatic advancement.

Table 32. Prioritized future work from analytical validation to formulation and safety.

Stage	Proposed work	Specific endpoints	Priority	Time horizon	Expected benefit	Decision criterion
Immediate analytical improvement	Repeat TFC and TAC calibration curves	Slope, intercept, residuals, linear range, accuracy, precision, LOD/LOQ where relevant	High	Short term	Improved analytical validity	Proceed only if acceptance criteria are predefined and met.
Immediate analytical improvement	Prepare independent extract batches	Yield, TPC, TFC, TAC, ABTS and DPPH across batches	High	Short term	Demonstration of reproducibility	Acceptable inter-batch variability and no unexplained outliers.
Immediate analytical improvement	Standardize ABTS and DPPH procedures	Control absorbance, blanks, incubation, temperature and solubility	High	Short term	Improved external comparability	Stable controls and reproducible dose-response curves.
Immediate analytical improvement	Compare solvents and extraction methods	Yield and normalized phytochemical/antioxidant outputs	Medium	Short term	Selection of a rational extraction process	Choose method using yield, activity, safety and scalability together.
Immediate analytical improvement	Perform LC-MS/MS or	Metabolite identification, fingerprints and marker candidates	High	Short to medium term	Chemical attribution and standardization	Reproducible fingerprint and selected

	HPLC-DAD profiling					quantitative markers.
Medium-term biological validation	Evaluate HepG2/HepaRG cytotoxicity	Viability, LDH, morphology, CC50 and non-cytotoxic range	High	Medium term	Definition of safe cellular concentrations	Only non-cytotoxic doses advance to protection assays.
Medium-term biological validation	Establish H2O2 or tert-BHP stress model	Stressor dose producing reproducible sublethal injury	High	Medium term	Validated cellular injury model	Stable assay window and responsive positive control.
Medium-term biological validation	Measure protection and redox endpoints	ROS, MDA, GSH/GSSG, SOD, CAT and GPx	High	Medium term	Evidence of intracellular antioxidant protection	Dose-responsive improvement without extract toxicity.
Medium-term biological validation	Measure hepatic injury endpoints	ALT, AST, LDH, morphology and apoptosis markers	High	Medium term	Evidence of reduced hepatocellular injury	Concordant improvement across multiple endpoints.
Medium-term biological validation	Model dose-response relationships	CC50, EC50, Emax and 95% confidence intervals	High	Medium term	Quantitative potency and selectivity	Curves must bracket the relevant response and pass model diagnostics.
Long-term formulation and safety	Standardize the extract using chemical markers	Marker concentration, fingerprint acceptance range and contaminants	Medium	Long term	Quality-control specifications	Specifications reproducible across production batches.
Long-term formulation and safety	Evaluate formulation stability	Light, temperature, humidity, oxidation and microbial quality	Medium	Long term	Shelf-life and storage conditions	Marker and functional stability within predefined limits.
Long-term formulation and safety	Conduct acute and repeated-dose oral safety studies	NOAEL, hematology, biochemistry, organ weights and histology	High	Long term	Systemic safety profile	No unacceptable target-organ toxicity at intended exposure.
Long-term formulation and safety	Evaluate in vivo hepatoprotective efficacy	ALT, AST, bilirubin, MDA, GSH, enzymes and histopathology	High	Long term	Physiological efficacy evidence	Predefined biochemical and histological protection after safety confirmation.

Long-term formulation and safety	Assess bioavailability and pharmacokinetics	Marker absorption, metabolites, exposure and elimination	Medium	Long term	Connection between formulation and systemic exposure	Quantifiable exposure to relevant markers or metabolites.
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III.11.7. Future research roadmap

Figure 37 summarizes the recommended sequence and the decision gates separating the three research levels. Analytical validation and chemical standardization should be completed before definitive cellular interpretation; biological safety and protection should be established before in vivo testing; and product-development activities should follow, rather than precede, evidence of safety and

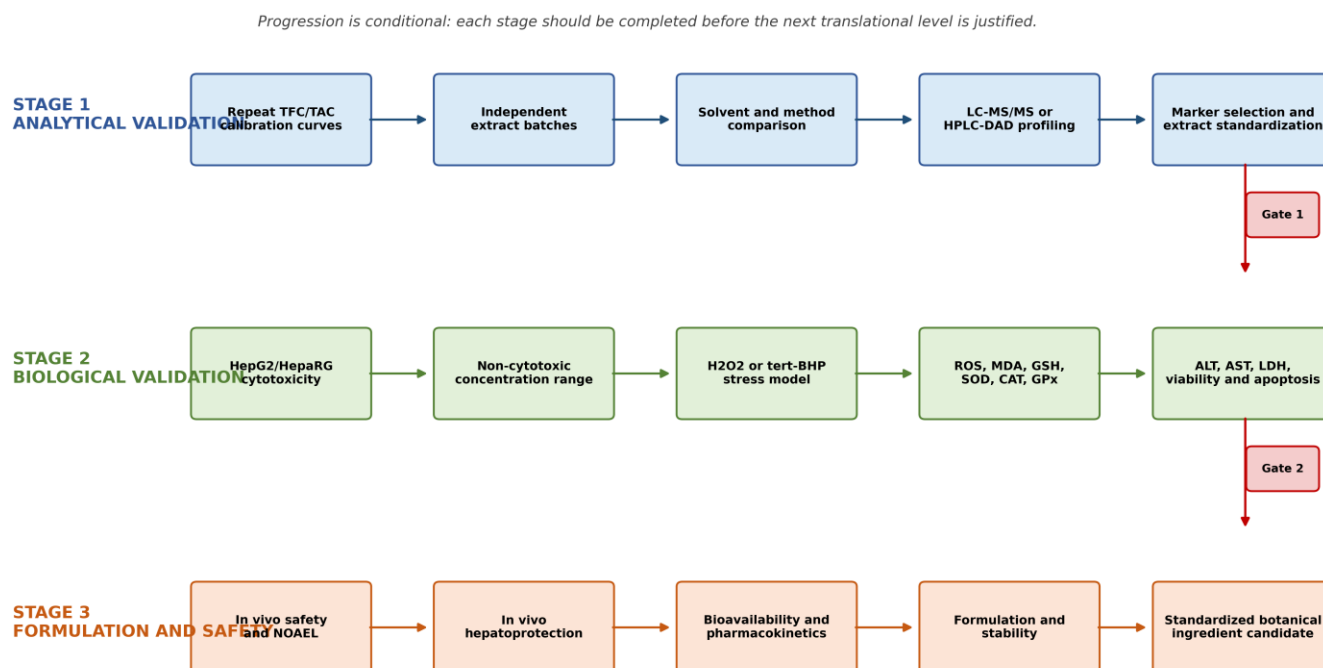


Figure 37. Prioritized future research roadmap for *C. monacantha*, progressing from analytical validation to biological confirmation and finally to formulation, safety and botanical-ingredient development.

III.11.8. Overall perspective

The present study should be positioned as a preliminary but internally coherent phytochemical and antioxidant evaluation. Its major strengths are the correction of analytical units, verification of inhibition calculations, transparent treatment of the non-reached DPPH IC50, and integration of qualitative and quantitative findings. Its principal limitations are the single extraction condition, absence of independent extraction batches, suboptimal linearity of some calibrations, lack of molecular identification, and absence of cellular, in vivo, toxicological and formulation validation. Addressing these limitations through the proposed staged program would transform a descriptive extract profile into a reproducible, biologically characterized and safety-supported research candidate.

Conclusion and Recommendations

Conclusion and Recommendations

This work characterized a single exploratory 50:50 ethanol-water macerate prepared from the aerial parts of *C. monacantha* collected in Bennacer, southeastern Algeria. The completed experiments comprised qualitative phytochemical screening, TPC, TFC, TAC, ABTS and DPPH assays. Hemolytic, antihemolytic and nutritional analyses were planned but could not be performed; consequently, the thesis provides no evidence on hemocompatibility, antihemolytic protection, lipids, proteins or vitamins.

Qualitative screening produced strong positive reactions for polyphenols, flavonoids and terpenoids (+++) and a moderate saponin response (++). TPC was 21.79 ± 0.50 mg GAE/g extract and was supported by an excellent gallic-acid calibration. TFC (8.42 ± 0.27 mg RE/g) and TAC (17.43 ± 0.42 mg AAE/g) showed low within-run variation but must be regarded as provisional estimates because the corresponding calibration R^2 values, 0.9595 and 0.9696, were insufficient for definitive quantitative validation.

The antioxidant response was assay-dependent. The extract showed a true interpolated ABTS IC₅₀ of 185.93 μ g/mL, approximately 4.47fold higher than that of ascorbic acid. In the DPPH assay, only 46.61% inhibition was reached at 1000 μ g/mL; the valid result is therefore IC₅₀ > 1000 μ g/mL. The extrapolated estimate was removed from integrated efficiency indices.

These findings define a moderate and method-dependent chemical antioxidant profile. They do not demonstrate hepatoprotection, cellular safety, oral safety, nutritional value or bioavailability. The 50:50 solvent ratio was a reasoned exploratory choice, not an optimized condition and not a direct reproduction of traditional Algerian aqueous preparations.

The contribution of this study lies in its transparent analytical interpretation: correction of standard-equivalent units, distinction between interpolated and non-reached IC₅₀ values, recognition of suboptimal calibration and explicit separation of completed work from planned but unperformed analyses. The findings justify further research only if the next stage begins with improved analytical quality.

Recommendations tailored to the observed moderate activity

- **Repeat TFC and TAC before biological escalation.** Rebuild the rutin and ascorbic-acid calibration curves with additional levels, independent preparations, replicate readings, residual analysis, recovery and predefined acceptance criteria.
- **Optimize extraction comparatively.** Compare water and several ethanol-water ratios, including the current 50:50 condition, using yield, chromatographic markers, TPC, ABTS/DPPH response, residual solvent and scalability. A more concentrated extract should not be assumed to be safer or more relevant.
- **Prepare independent extraction batches.** Quantify inter-batch variation rather than relying only on triplicate readings from one extract.

- **Prioritize chemical identification.** Use HPLCDAD and LCMS/MS to select marker compounds and establish a reproducible fingerprint.
- **Address the weak DPPH result realistically.** Rather than seeking a stronger claim, investigate whether solvent compatibility, kinetics or specific polar constituents explain the more favourable ABTS response.
- **Complete the omitted safety work prospectively.** Any hemolytic assay involving human erythrocytes must receive prior ethics approval and informed consent. Cellular viability should precede protection experiments.
- **Generate the missing compositional dossier.** Determine lipids, proteins, vitamins, minerals, moisture, ash, microbiological quality, contaminants and relevant antinutritional constituents before nutritional or product claims are considered.
- **Use a staged liver-directed program.** Only after analytical revalidation and cytotoxicity testing should Noncytotoxic concentrations be assessed in hepatic oxidative-stress models. In vivo studies should follow, not precede, cellular safety and reproducible chemical standardization.
- **Develop a concrete but conditional valorization prototype.** If the preceding decision gates are met, prepare a marker-standardized dry extract for a simple capsule or sachet prototype. Define residual ethanol, moisture, marker range, microbial limits, packaging and accelerated/real-time stability. This is a development strategy, not a recommendation for immediate commercialization.
- **Integrate the Algerian regulatory pathway.** Product classification, permitted claims, quality specifications and authorization requirements must be determined with the competent Algerian authorities. International guidance should remain supportive rather than substitutive.

In conclusion, *C. monacantha* yielded a scientifically interesting but moderate and incompletely validated chemical antioxidant profile. Its valorization potential is conditional on extraction optimization, analytical revalidation, marker-based standardization, completion of safety and nutritional studies, and biologically relevant confirmation. At the current stage, the appropriate output is a carefully characterized research extract, not a liver-support product.

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ABSTRACT

التوصيف الكيميائي النباتي والقدرة المضادة للأكسدة للمستخلص الهيدروإيثانولي لنبات *Cornulaca monacantha* Delile: آفاق تطويره كمكوّن نباتي داعم لوظائف الكبد

الملخص

هدفت هذه الدراسة إلى توصيف التركيب الكيميائي النباتي وتقييم القدرة المضادة للأكسدة لمستخلص هيدروإيثانولي محضّر من الأجزاء الهوائية لنبات *Cornulaca monacantha* Delile المجموع من جنوب شرق الجزائر. أظهر التحري الكيميائي النباتي النوعي تفاعلات إيجابية قوية لمتعددات الفينول والفلافونويدات والتربينويدات (+++)، مع تفاعل إيجابي متوسط للصابونينات (++). بلغ المحتوى الكلي لمتعددات الفينول 21.79 ± 0.50 ملغ مكافئ حمض الغاليك/غ من المستخلص، بينما بلغ المحتوى الكلي للفلافونويدات 8.42 ± 0.27 ملغ مكافئ روتين/غ، وبلغت القدرة الكلية المضادة للأكسدة 17.43 ± 0.42 ملغ مكافئ حمض الأسكوربيك/غ. وأظهرت معاملات الاختلاف المنخفضة، التي تراوحت بين 2.31% و3.20%، تكرارية تحليلية جيدة. في اختبار ABTS، بلغ تركيز الشيط النصفى للمستخلص 185.93 ميكروغرام/مل مقابل 41.62 ميكروغرام/مل لحمض الأسكوربيك. أما في اختبار DPPH، فلم يصل المستخلص إلى 50% من التثبيط ضمن مجال التراكيز المدروسة؛ ولذلك سُجّلت قيمة IC_{50} علمياً على أنها أكبر من 1000 ميكروغرام/مل. وتؤكد هذه النتائج أن الاستجابة المضادة للأكسدة تعتمد على طبيعة الاختبار، مع استجابة أكثر وضوحاً في اختبار ABTS مقارنة باختبار DPPH. وعموماً، أظهر المستخلص بروفائلاً كيميائياً نباتياً متناسقاً وقدرة متوسطة مضادة للأكسدة. غير أن النتائج الحالية، المتحصل عليها في أنظمة كيميائية خالية من الخلايا، لا تثبت فعالية واقية للكبد أو سلامة خلوية أو فموية. لذلك، تبقى دراسات التوصيف الكيميائي باستخدام LC-MS/MS، واختبارات السمية الخلوية، ونماذج الإجهاد التأكسدي على الخلايا الكبدية ضرورية قبل التفكير في تطوير هذا المستخلص كمكوّن نباتي معياري داعم لوظائف الكبد.

الكلمات المفتاحية: *Cornulaca monacantha*؛ التحري الكيميائي النباتي؛ المركبات الفينولية؛ النشاط المضاد للأكسدة؛ دعم وظائف الكبد.

Phytochemical Characterization and Antioxidant Potential of the Hydroethanolic Extract of *Cornulaca monacantha* Delile: Prospects for Its Development as a Botanical Ingredient for Liver-Function Support

ABSTRACT

This study investigated the phytochemical composition and antioxidant potential of a hydroethanolic extract prepared from the aerial parts of *Cornulaca monacantha* Delile collected in south-eastern Algeria. Qualitative phytochemical screening revealed strong positive reactions for polyphenols, flavonoids and terpenoids (+++), together with a moderate positive reaction for saponins (++). The total phenolic content was 21.79 ± 0.50 mg gallic acid equivalents per gram of extract, while the total flavonoid content was 8.42 ± 0.27 mg rutin equivalents per gram of extract and the total antioxidant capacity was 17.43 ± 0.42 mg ascorbic acid equivalents per gram of extract. The low coefficients of variation, ranging from 2.31% to 3.20%, indicated good analytical repeatability. In the ABTS assay, the extract exhibited an IC₅₀ of 185.93 µg/mL, compared with 41.62 µg/mL for ascorbic acid. In contrast, the extract did not reach 50% DPPH inhibition within the tested concentration range; its IC₅₀ was therefore correctly reported as >1000 µg/mL. These findings demonstrate an assay-dependent antioxidant response, with a markedly stronger response in the ABTS assay than in the DPPH assay. Overall, the extract exhibited a coherent phytochemical profile and moderate antioxidant potential. However, the current findings, obtained using cell-free chemical systems, do not demonstrate hepatoprotective efficacy, cellular safety or oral safety. Further LC-MS/MS characterization, cytotoxicity testing and oxidative-stress studies in hepatic cell models are therefore required before its development as a standardized botanical ingredient for liver-function support can be considered.

Keywords: *Cornulaca monacantha*; phytochemical screening; phenolic compounds; antioxidant activity; liver-function support.

Caractérisation phytochimique et potentiel antioxydant de l'extrait hydroéthanolique de *Cornulaca monacantha* Delile : perspectives de valorisation comme ingrédient botanique destiné au soutien de la fonction hépatique

RÉSUMÉ

Cette étude a porté sur la caractérisation phytochimique et l'évaluation du potentiel antioxydant d'un extrait hydroéthanolique préparé à partir des parties aériennes de *Cornulaca monacantha* Delile, récoltées dans le Sud-Est algérien. Le criblage phytochimique qualitatif a révélé des réactions fortement positives pour les polyphénols, les flavonoïdes et les terpénoïdes (+++), ainsi qu'une réaction modérément positive pour les saponines (++). La teneur totale en composés phénoliques était de $21,79 \pm 0,50$ mg équivalents d'acide gallique/g d'extrait, tandis que la teneur totale en flavonoïdes était de $8,42 \pm 0,27$ mg équivalents de rutine/g d'extrait et que la capacité antioxydante totale atteignait $17,43 \pm 0,42$ mg équivalents d'acide ascorbique/g d'extrait. Les faibles coefficients de variation, compris entre 2,31 % et 3,20 %, ont indiqué une bonne répétabilité analytique. Dans le test ABTS, l'extrait a présenté une IC₅₀ de 185,93 µg/mL, contre 41,62 µg/mL pour l'acide ascorbique. En revanche, l'extrait n'a pas atteint 50 % d'inhibition du DPPH dans la gamme de concentrations étudiée ; son IC₅₀ a donc été correctement rapportée comme étant supérieure à 1000 µg/mL. Ces résultats mettent en évidence une réponse antioxydante dépendante de la méthode, avec une réponse nettement plus marquée dans le test ABTS que dans le test DPPH. Globalement, l'extrait a présenté un profil phytochimique cohérent et un potentiel antioxydant modéré. Toutefois, les résultats actuels, obtenus dans des systèmes chimiques acellulaires, ne démontrent ni une efficacité hépatoprotectrice ni une sécurité cellulaire ou orale. Une caractérisation par LC-MS/MS, des essais de cytotoxicité et des études du stress oxydatif sur des modèles cellulaires hépatiques sont donc nécessaires avant d'envisager son développement comme ingrédient botanique standardisé destiné au soutien de la fonction hépatique.

Mots-clés : *Cornulaca monacantha* ; criblage phytochimique ; composés phénoliques ; activité antioxydante ; soutien de la fonction hépatique.