Unveiling the Allelopathic Potential of Cornulaca Monacantha Del.: Phytochemical Profiling and Bioherbicidal Activity Against Chenopodium Murale

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Abstract
This study investigates the herbicidal potential of Cornulaca monacantha Del., a halophytic plant, against the weed Chenopodium murale, alongside comprehensive phytochemical profiling and antioxidant activity evaluation. Methanolic extracts of C. monacantha were subjected to quantitative phytochemical analysis, revealing high concentrations of total phenolics (46.3 mg GAE /g DE) and flavonoids (44.15 mg QE /g DE), with significantly lower alkaloid content (7.38 mg NE/g DE). LC-MS analysis identified p-Coumaric acid as the predominant compound (91.32%) in the 20% methanol fraction, with its relative abundance decreasing as methanol concentration increased. Bioherbicide assays demonstrated remarkable efficacy, with the crude extract and 20% methanol fraction achieving complete (100%) inhibition of C. murale, comparable to a commercial synthetic herbicide. The extract also exhibited significant antioxidant activity (IC_{50} = 10.498 mg/mL) in the DPPH assay, though less potent than ascorbic acid (IC_{50} = 0.265 mg/mL). These findings strongly correlate the phytotoxic activity of C. monacantha with its high phenolic content, particularly p-Coumaric acid. The study concludes that C. monacantha represents a promising source of natural herbicides, warranting further investigation into the isolation of active compounds and field-scale validation for integrated weed management strategies.

Keywords: allelopathy; antioxidant activity; bioherbicide; Cornulaca monacantha; p-Coumaric acid; phytochemical profiling;

1- Introduction

Weed competition ranks among the primary biological limitations to crop production, causing considerable annual harvest reductions and thereby threatening worldwide food supplies (Oerke, 2006). Chemical herbicides have long served as the foundation of modern weed control because they are both efficient and economically advantageous. Nevertheless, their widespread and repeated application over many years has generated major drawbacks: the selection of resistant weed populations (Heap, 2014), contamination of soil and water resources, and increasing concerns regarding toxicological effects on non-target organisms, including humans (Carvalho, 2017). This worrying situation has led to a major change in the way the farming industry thinks about the environment, with the development of eco-friendly options being a top priority (Dayan and al., 2012). A wide diversity of secondary metabolites—including phenolic compounds, flavonoids, and alkaloids—is synthesized by plants. These molecules frequently contribute to both defensive mechanisms and allelopathic interactions, the latter referring to the chemical-mediated effects of one plant species on the establishment, development, or reproductive success of adjacent plants (Farooq et al., 2011). These natural compounds can interfere with vital physiological processes in target weeds, including cell division, membrane integrity, and photosynthesis, offering a potent yet biodegradable mechanism for weed control (Scavo and Mauromicale, 2021). The exploration of plant species, especially those adapted to harsh environments, for novel phytotoxic compounds is therefore a key strategy in bioherbicide research.

Cornulaca monacantha Del. (Amaranthaceae) is a salt-tolerant bush that is indigenous to the arid and semi-arid regions of North Africa and the Near East. Renowned for its resilience in saline deserts, it has traditionally been used in folk medicine to treat ailments like inflammation or diabetes, which suggests that it contains many

bioactive constituents (Hussien et al., 2023). Earlier phytochemical research on C. monacantha has chiefly centred on its therapeutic characteristics, verifying the existence of valuable metabolites like saponins, flavonoids, and phenolic acids, which are recognised to contribute to its antioxidant and antimicrobial actions (Kandil et al., 2001; Boussadia et al., 2024).While the biocidal potential of related halophytic species in the Amaranthaceae family, such as Salsola and Atriplex, has been documented for weed and pest management purposes (El-Keblawy& Al-Rawai, 2007; Khan et al., 2024), the specific herbicidal potential of Cornulaca monacantha has not yet been scientifically validated against particular weeds and remains largely unexplored. While a few preliminary studies have suggested allelopathic effects with plant extracts from arid environments (Motmainna et al., 2023), there is a lack of targeted research linking the phytochemical profile of C. monacantha to its efficacy against specific problematic weeds. Chenopodium murale (common name: Nettle-leaved Goosefoot) is a weed that is found all over the world. It is a type of plant that grows quickly and takes over other plants. It is known to grow on many different types of crops and can make the amount of food that grows from these crops go down a lot (Raza and al., 2023).

In view of this situation, the current investigation was conceived to methodically examine the potential of Cornulaca monacantha to inhibit the growth of Chenopodium murale. The specific objectives were to: (1) Look closely at the chemicals in plants, including how much of each type of chemical there is, and find out what the main chemicals are using a special machine; (2) Test how well the plant extract can stop the DPPH problem in a test tube; and (3) Find out how well different types of plant extract from C. monacantha can stop the growth of C. murale. By integrating chemical profiling with bioactivity screening, this research aims to lay the scientific groundwork for the development of C. monacantha as a natural, sustainable tool for integrated weed management.

2- Materials and Methods

Plant Material Collection and Preparation

Fresh specimens of C. monacantha ex Spreng were collected in autumn 2021 from the El Oued region (33°21′40″N, 6°51′38″E) in southeastern Algeria. Authentication of the plant material was carried out at INRAA Touggourt according to the principles of taxonomy. Depositing of a voucher specimen at the laboratory was done under the reference number INRAA 55.

The aerial parts were thoroughly washed with distilled water to remove dust and debris, then air-dried at ambient temperature (25 ± 2°C) in the shade for two weeks to prevent photo degradation of bioactive compounds. The dried material was finely powdered using an electric grinder and passed through a 40-mesh sieve to ensure uniform particle size. The resulting powder was stored in airtight containers at 4°C until further processing.

Sequential Extraction Procedure

The plant material, which weighed 50 grammes, was extracted using a Soxhlet apparatus (Gerhardt Analytical Systems, Germany) and the process was done in sequence. Initially, extraction was carried out with 99% pure hexane (Sigma-Aldrich) for two hours to isolate non-polar phyto-constituents. The residual plant material was then extracted with methanol (analytical grade, Sigma-Aldrich) under identical conditions for a further two hours to isolate polar compounds.

Each extraction cycle was continued until the solvent in the syphon tube appeared colourless, indicating exhaustive extraction. The solvent fractions were then concentrated using a rotary evaporator (Büchi Rotavapor R-300, Switzerland) at 40°C to obtain crude extracts. The process of drying under a gentlest ream of nitrogen gas en sured the complete removal of residual solvents.

The dry weight of the original plant material was used to calculate the yield of extract. Then, we stored the crude extract in sterile, amber-coloured glass vials at 4°C in the dark to stop it from degrading any more before doing some more phytochemical, antioxidant, and herbicidal analyses.

A preliminary qualitative phytochemical screening was conducted using standard methods to identify the major classes of secondary metabolites present in the methanolic extracts from C. monacantha. All tests were performed in triplicate for reproducibility. Analytical-grade chemicals and reagents (Sigma-Aldrich, USA) were used throughout the study.

Phytochemical screening: Quantification of Polyphenols, Flavonoids and Alkaloids

The total polyphenol and flavonoid content of the methanolic extracts from C. monacantha seeds and leaves was quantified following a phytochemical screening. All analyses were performed in triplicate using spectrophotometric methods (Lakhdari and al., 2024). Assessment of the presence of alkaloids in the hexane and methanolic extracts of C. monacantha was conducted using the Dragen dorff's reagent colorimetric method, following established protocols (Pender and al., 2024).

Liquid Chromatography-Mass Spectrometry analysis

The phenolic compounds were identified using an LC-MS system. A vacuum degasser, an autosampler and a binary pump with a maximum pressure of 400 bar (Agilent 1260, Agilent Technologies, Germany) were used. This was provided with a reverse-phase C18 analytical column of 4.6 x 100 mm and 3.5 µm particle size (Zorbax Eclipse XDB C18). The setting of the DAD detector was to a scanning range of 200-400 nm. The temperature of the column was set at 25°C. The composition of mobile phase B was milli-Q water with 0.1% formic acid, while mobile phase A was 0.2% methanol. The flow rate was maintained at 0.4 ml/min. The improved elution gradient was as follows: 0–2 min: A 95%; 2–15 min: A 95%; 15–18 min: A 5%; 18–20 min: A 5%; 20–30 min: A 95%; then return to initial conditions. A 2 µl volume was injected for each sample and the peaks were monitored at 280 nm. Phenolic compounds were identified based on their retention times and the UV spectra of the phenolic chromatogram. The pure standards were myricitrin, quercetin, p-coumaric acid, naringenin, and syringic acid.

Identification was performed by the retention times of the standards being compared with those of the extracts. A calibration curve was obtained for the quantitative analysis by plotting the peak areas against different concentrations for each identified compound at 280 nm.

Fractionation

Methanol filtrates were combined. They were concentrated under vacuum. The filtrates were fractionated using a reverse-phase silica gel column. The fractions were separated using different concentrations of methanol (20%, 40%, 60%, 80%, and 100%) to obtain different types of phenolic fractions, which were then evaporated separately (Susanti and al., 2024).

Formulation

A formulated herbicide was prepared by mixing the plant extracts, combined with vegetable oil, facilitate the absorption of nutrients by increasing the permeability of the epicuticular waxes. The formulation contained 2% extract, 3.4% vegetable oil, 93.10% distilled water, 0.67% Tween 20, 0.5% ethanol, and 0.33% Sorbiton oil (Ben Kaab and al.,2020).

Bioassay of Plant Extracts on Weed Germination

To evaluate the herbicidal potential of C. monacantha extracts against the weed C. murale, a germination bioassay was conducted using various plant extract fractions, as detailed in previous sections. Seeds of the C. murale plant were obtained from the eastern region of El Oued and stored for later use. Prior to use, the seeds must be sterilised by immersing them in a 0.5% sodium hypochlorite solution for 20 minutes. They should then be rinsed with distilled water three times to remove any residual chemicals.

Each treatment consisted of a combination of plant extract fractions, a chemical herbicide (used as a positive control), and distilled water (as a negative control). In each case, 15 seeds were placed in Petri dishes, with three replicates (three Petri dishes per treatment) prepared for each test group, including the plant extract, chemical herbicide, and distilled water. The Petri dishes were randomly placed in a dark room to ensure consistent experimental conditions.

The germination rate was recorded after 10 days, and the number of germinated seedlings was counted for each treatment. The germination index (GT) was calculated using the following equation (Chiapusio and al.,1997):

$$GT = \frac{NT \times 100}{N}$$

Where:

- **NT** is the number of germinated seeds for each treatment at the end of the assay.
- **N** is the total number of seeds used in the assay (15 seeds per Petri dish).

This germination test provides a reliable measure of the herbicidal activity of the plant extracts, as the germination index (GT) is a commonly used and widely accepted parameter for evaluating the effects of bioactive compounds on seedling development.

Antioxidant activity

The antioxidant activity of the methanolic extract of C. monacantha was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a widely used method for assessing the free radical scavenging activity of plant extracts (Baschieri and Amorati,2021). DPPH is a stable free radical that reacts with antioxidants, leading to a characteristic color change from purple to yellow. This reaction can be quantitatively measured by spectrophotometry.

For the assay, 1 mL of the methanolic extract (at various concentrations) was added to 3 mL of a DPPH solution (0.1 mM in methanol). The mixture was then incubated in the dark at room temperature for 30 minutes. The absorbance of the resulting solution was measured at 517 nm using a UV-Vis spectrophotometer. A decrease in absorbance at 517 nm indicates the scavenging of DPPH radicals by the extract.

Statistical analysis

The software IBM SPSS Statistics (Statistical Package for the Social Sciences) v. 26 for Windows was utilised to perform parametric (ANOVA followed by Tukey's HSD test). The disparities between the means of the individual groups were deemed to be of significance if $p < 0.05$.

3 Results

Phytochemical Analysis

Phytochemical quantification of the methanolic extract of Cornulacamonacantha confirmed the presence of major secondary metabolite classes, notably phenolics, flavonoids, and alkaloids. The extract was particularly rich in phenolic compounds, with a total content of 46.3 ± 0.10 mg GAE /g DE (Gallic acid equivalent per gram of dry extract). The total flavonoid content was also substantial at 44.15 ± 0.12 mg QE /g DE (Quercetin equivalent per gram of dry extract). In contrast, the total alkaloid content was significantly lower, quantified at 7.38 ± 0.275 mg NE/g DE (Nicotine equivalent per gram of dry extract) (Table 1).

Table 1. Phytochemical analysis of Cornulaca monacantha extract.

Total	Extracts of Cornulaca monacantha	Curve Equation	R^2

Total phenolics (mg GAE/g DE)	46.3±0.10	ABS = 0.018x-0.1843	0.999
Total flavonoids (mg QE/g DE)	44.15±0.12	ABS = 0.0015x+0.0123	0.999
Total alkaloids (mg NE/g DE)	7.38±0. 275	ABS = 0.0441x + 0.1002	0.999

GAE: Gallic acide equivalent. QE: Quercetin equivalent. NE: Nicotine equivalent. DE: Dry extract.

The high R^2 values (0.999 for all metabolites tested) suggest that the linear models used to estimate concentrations of different secondary metabolites from absorbance measurements are very accurate. The levels of three types of secondary metabolites in C. monacantha were compared using the results of a one-way analysis of variance (ANOVA) with post-hoc Tukey's test. These are polyphenols, alkaloids and flavonoids. The analysis of variance results shows that there is a significant effect of the secondary metabolite type on the production of C. monacantha ($F = 4345.487$, $p < 0.0001$).

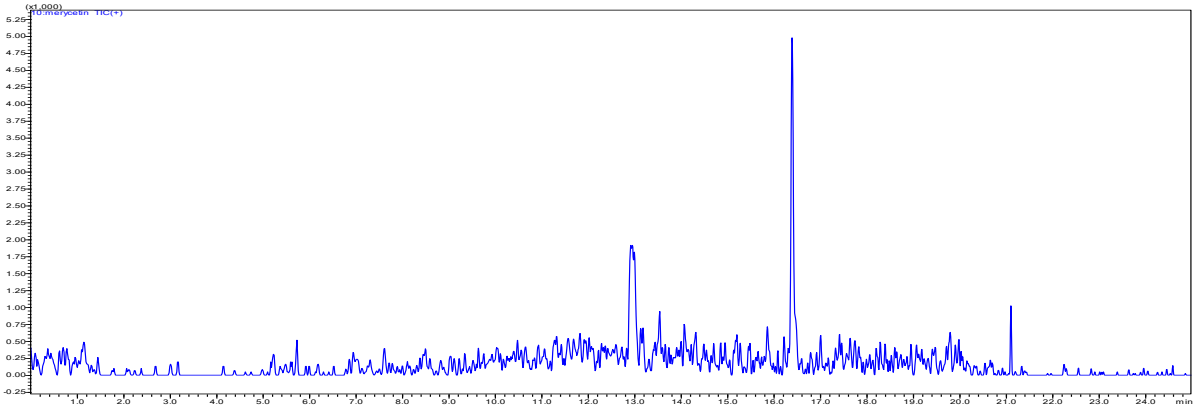
Liquid Chromatography-Mass Spectrometry Analysis

Liquid Chromatography-Mass Spectrometry (LC-MS) was employed for a detailed characterization of the extract's composition across a gradient of methanol concentrations (20–100%). The analysis identified a range of bioactive compounds, with their distribution heavily influenced by solvent polarity (Table 2).

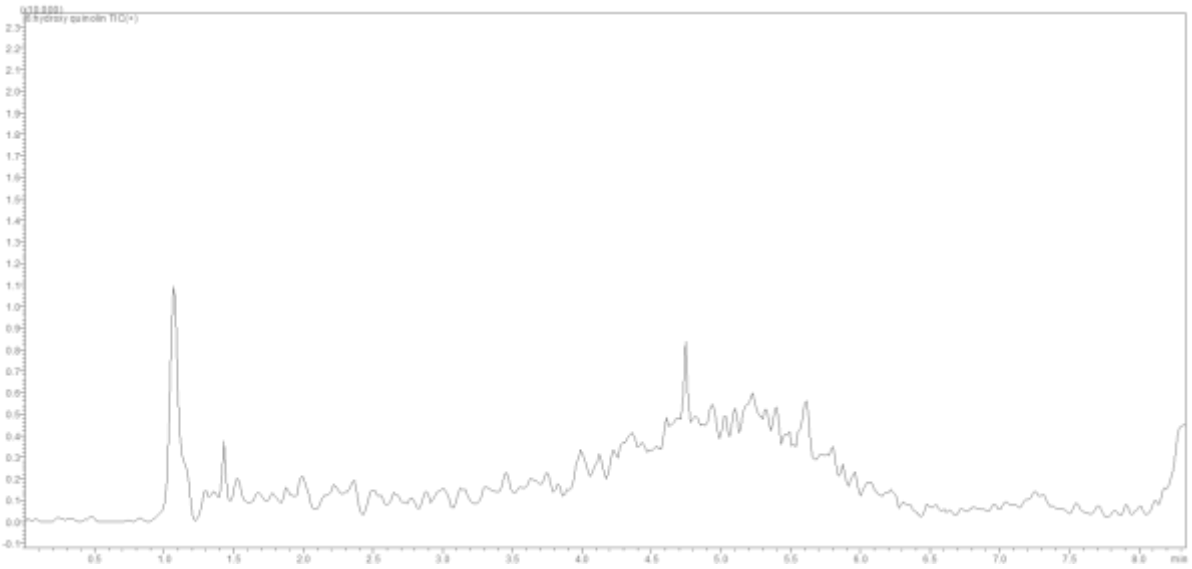
Table 2. Comparative LC–MS Summary of Methanol Extracts (20–100%) of C. monacantha

Compound	20% MeOH	40% MeOH	60% MeOH	80% MeOH	100% MeOH	Class
p-Coumaric acid	91.32%	79.44%	73.02%	64.25%	18.01%	Phenolic acid
Kaempferol	2.09%	11.52%	12.10%	13.33%	29.24%	Flavonol
β-Carotene	2.17%	6.21%	8.57%	15.10%	37.62%	Carotenoid
Hesperetin	0.72%	0.56%	1.05%	2.56%	14.93%	Flavanone
Quercetin	0.02%	0.09%	0.08%	0.10%	0.40%	Flavonol
Salicylic acid	0.01%	0.19%	0.17%	0.23%	0.39%	Phenolic acid
Myricetin	0.03%	0.02%	0.02%	-	0.02%	Flavonol
Thymol	3.64%	2.87%	3.00%	2.50%	0.13%	Phenolic monoterpenoid

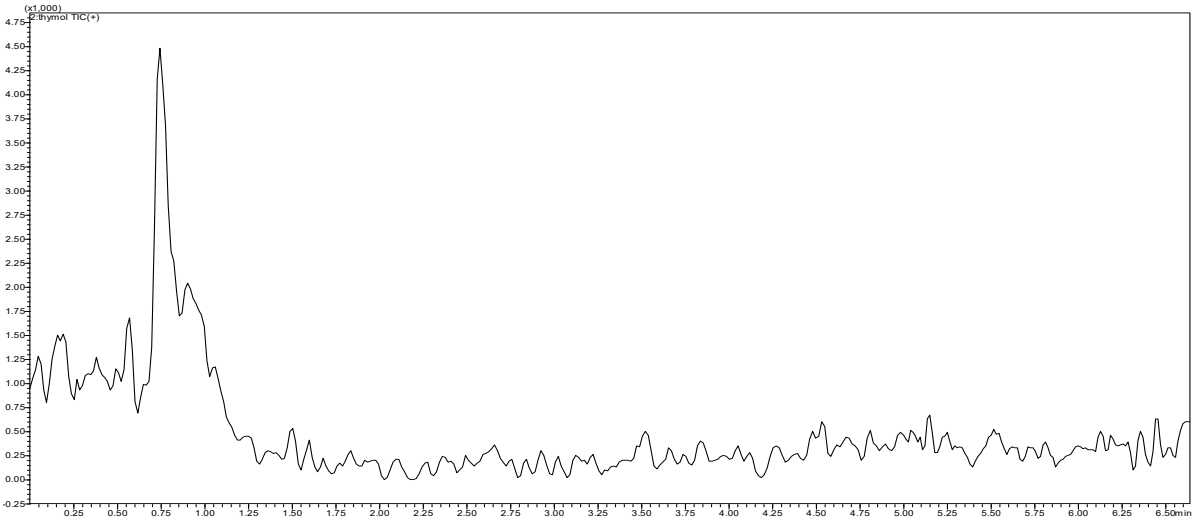
A striking finding was the extreme predominance of p-Coumaric acid in the 20% methanol extract, where it constituted 91.32% of the detected compounds. Its relative abundance decreased progressively as methanol concentration increased. Conversely, the carotenoid β-Carotene and the flavonol Kaempferol exhibited a strong positive correlation with solvent polarity, reaching their highest abundances of 37.62% and 29.24%, respectively, in the 100% methanol fraction. Hesperetin also showed a marked increase in the pure methanol extract. This solvent-dependent partitioning highlights the differential solubility of these compounds and is visually summarized in the LC-MS chromatograms (Figure 1).



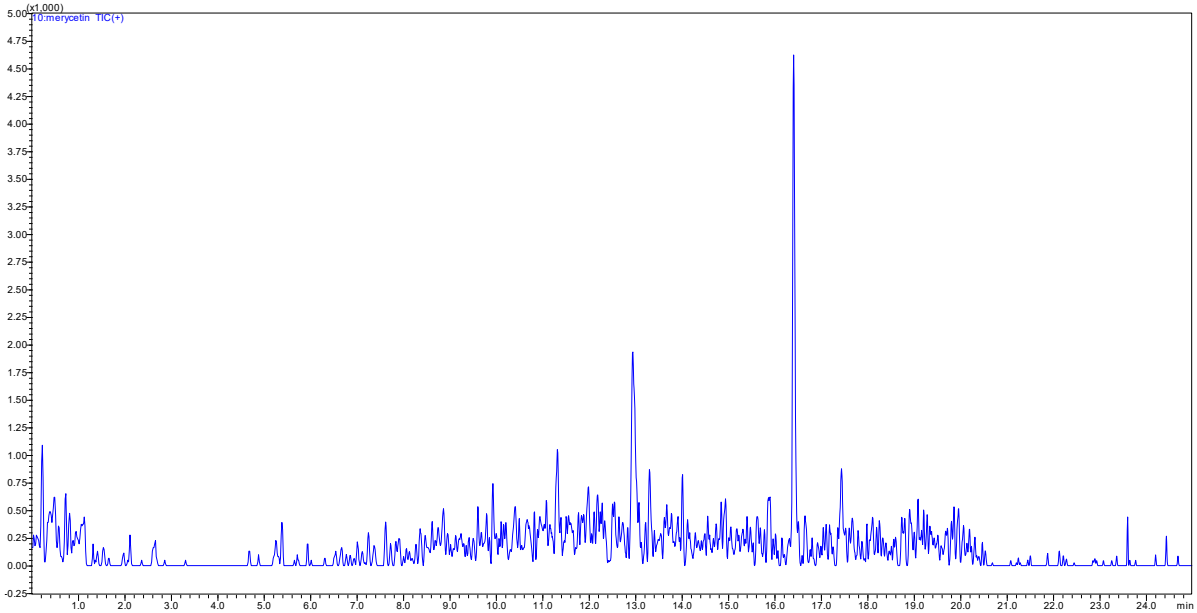
(A)



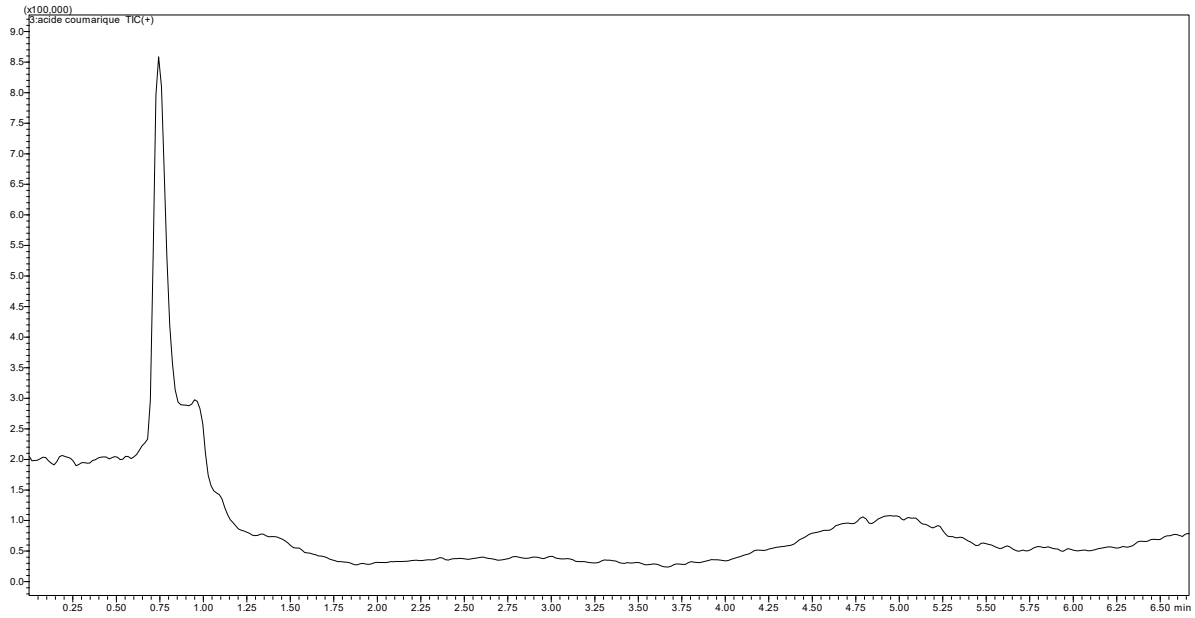
(B)



(C)



(D)



(E)

Figure 1. LC–MS chromatogram of Cornulaca monacantha methanol extracts (20–100%)
Fraction 20% ; (B) Fraction 40% ; (C) Fraction 60% ; (D) Fraction 80% ; (E) Fraction 100%

Potent Bioherbicidal Efficacy Against Chenopodium murale

The bioherbicidal potential of C. monacantha extracts was evaluated on the weed Chenopodium murale. The results demonstrated significant inhibitory effects across different formulations (Table 3). Notably, the commercial herbicide (positive control), the **crude methanolic extract**, and the **20% methanol fraction** achieved complete inhibition of plant growth (**100%**). The formulation without the crude extract showed 72.2% inhibition, indicating an inherent effect of the adjuvant mixture. Among the other fractions, the 40% methanol fraction exhibited the highest activity (**82.6%**), while fractions from 60% to 100% methanol showed moderate but significant inhibitory rates ranging from 70.8% to 77.4%. The distilled water control exhibited a 33.2% inhibition, reflecting natural mortality or experimental conditions.

Table 3. Inhibitory activity of C. monacantha methanol extract against Chenopodium murale.

Extracts	Total inhibition (%)
Distilled water (control)	33.2±8.167a
Herbicide	100±0.000c
Formulation + crude extract	100±0.000c
Formulation without crude extract	72.2±7.120b
Formulation + Fraction 20%	100±0.000c
Formulation + Fraction 40%	82.6±16.072bc
Formulation + Fraction 60%	70.8±8.871b
Formulation + Fraction 80%	77.4±10.188b
Formulation + Fraction 100%	74.8±21.23b

Antioxidant activity

The ability of a substance to scavenge or neutralise free radicals, which are molecules with unpaired electrons, is measured by the DPPH assay. In this assay, DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that reacts with antioxidants by donating hydrogen atoms or electrons. This leads to a colour change from purple to yellow as the DPPH radical is reduced. The IC_{50} value represents the concentration of the substance required to inhibit 50% of the DPPH radicals, thereby indicating its antioxidant activity.

Our results show that the methanolic extract of Cornulaca monacantha had antioxidant activity with an IC50 value of 10.498 mg/mL, indicating its antioxidant potential. Ascorbic acid, a well-known antioxidant, had a lower IC_{50} value of 0.265 mg/mL, suggesting that it is a more potent antioxidant in this assay.

4 Discussion

Phytochemical Analysis

The quantitative phytochemical analysis revealed a high concentration of phenolic compounds (46.3 mg GAE /g DE) and flavonoids (44.15 mg QE /g DE), significantly surpassing the alkaloid content (7.38 mg NE/g DE). High levels of phenolic compounds are commonly observed in halophytic species, where they play a functional role in physiological acclimation to environmental constraints, including high salinity and water deficit (Ksouri and al., 2008; Anwar et al., 2023). The high levels of flavonoids and phenolic compounds are particularly relevant, as these metabolite classes are often associated with antioxidant and phytotoxic activities (Macías et al., 2007).

Liquid Chromatography-Mass Spectrometry Analysis
A deeper, more nuanced understanding of how extraction solvent polarity influences the yield of specific bioactive compounds was offered by the LC-MS analysis. The remarkable dominance of p-Coumaric acid (91.32%) in the 20% methanol extract is a critical finding. Previous investigations have established that p-coumaric acid, a common phenolic compound, possesses allelopathic and phytotoxic activities. Its mode of action includes disruption of mitotic processes, alterations in membrane integrity, and interference with plant hormone regulation in sensitive species (Barkosky & Einhellig, 1993; Li and al., 2010). This finding directly explains the 100% inhibition achieved by the 20% methanol fraction in our bioherbicide assays. The efficacy of this fraction was on par with a commercial synthetic herbicide, underscoring its potent phytotoxicity. As the methanol concentration increased, the extract's composition shifted towards more non-polar compounds like β-Carotene and Kaempferol. The concurrent decline in the herbicidal inhibition efficacy of the 40%-100% fractions suggests that while these compounds may contribute to overall bioactivity, p-Coumaric acid is likely a primary driver of the strong phytotoxic effect observed in the crude extract and the 20% fraction. This structure-activity relationship, mediated by solvent selectivity, provides a strategic insight for optimizing extraction protocols aimed at maximizing herbicidal potency.

Potent Bioherbicidal Efficacy Against Chenopodium murale
The bioherbicidal assay results were compelling. The complete inhibition of C. murale by the crude extract and the 20% fraction highlights their potential as natural weed management solutions. It is noteworthy that the adjuvant formulation alone exhibited 72.2% inhibition, which must be accounted for when interpreting the activity of the fractionated extracts. However, the significant boost to 100% inhibition upon the addition of the crude or 20% fraction clearly demonstrates a synergistic effect where the plant's bioactive compounds are the decisive agents. The mode of action likely involves multiple pathways, a common trait of natural phytotoxins. The high phenolic content, particularly p-Coumaric acid, could induce oxidative stress by generating reactive oxygen species (ROS), damaging cellular structures, and inhibiting key enzymes involved in germination and growth (Scavo and al., 2018).

Antioxidant activity
In addition to its herbicidal potential, the methanolic extract of C. monacantha exhibited considerable antioxidant activity, with a DPPH radical scavenging IC_{50} value of 10.498 mg/ml. This activity is rationally attributed to its high levels of phenolic and flavonoid compounds, which are renowned for their ability to donate hydrogen atoms and stabilize free radicals (Rice-Evans et al., 1997). The lower radical-scavenging efficiency of the methanolic extract relative to purified ascorbic acid is not surprising, given that the crude plant preparation represents a complex mixture where only a portion of the constituents contribute to antioxidant activity. The demonstrated activity is significant in its own right and suggests that the extract could also possess protective properties against oxidative stress, which is often linked to its mechanism of phytotoxicity—whereby pro-oxidant actions in target weeds lead to cellular damage (Weir and al., 2004).The integration of phytochemical profiling with bioactivity screening in this study establishes a clear link between the chemical constitution of C. monacantha and its observed physiological effects. The high concentration of phenolics, especially the solvent-specific abundance of p-Coumaric acid, is strongly correlated with its potent bioherbicidal activity. These findings not only validate the traditional use of C. monacantha but also provide a solid scientific foundation for its development as a natural, eco-friendly alternative to synthetic herbicides. Future work should focus on the isolation and individual efficacy testing of p-Coumaric acid and other major compounds, field trials to validate greenhouse results, and toxicological studies to ensure environmental and non-target species safety.

5 Conclusions
This study establishes Cornulaca monacantha as a promising source of natural herbicides, driven by its high concentration of bioactive phenolics. The remarkable finding is that the 20% methanolic fraction, dominated by p-Coumaric acid, achieved complete inhibition of Chenopodium murale, rivaling a commercial herbicide. This direct link between a specific compound and potent phytotoxicity offers a clear and sustainable pathway for developing effective, natural weed control solutions, warranting further investigation into its application.

Author Contributions
Conceptualization: W.L. and A.D; Data curation: A.F; Formal analysis: M.S; Funding acquisition; Investigation: W.L. and M.S; Methodology: A.F and W.L; Project administration; Resources; Software; Supervision: W.L. and M.S; Validation: F.B., H.H.,D.M., I.G. and F.D; Visualization; Writing—original draft: A.F. and D.M; Writing—review and editing: W.L., M.S. and A.D.
All authors read and approved the final manuscript.

Ethical Approval (if applicable)
Not applicable.

Acknowledgments
Thanks go to Mr. Neghmouche Nacer Salah for their support and assistance to us in this article

Conflict of Interest
The authors declare no conflicts of interest related to the content of this article.

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