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Herbicidal potential of *Argemone mexicana* L. extracts to control *Ageratum conyzoides* L.

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ABSTRACT

We evaluated the herbicidal potential of *Argemone mexicana* extracts against *Ageratum conyzoides*, a problematic agricultural weed. Aqueous extracts were prepared from dried *A. mexicana* plant material and tested at concentrations of 10,50,100 and 200 mg/ml. Laboratory bioassays assessed seed germination, seedling growth, biomass accumulation, photosynthetic pigments and protein content. Bioactive compounds were identified using GC-MS analysis. Field trials evaluated weed control efficacy under natural conditions using a randomized complete block design. Extract significantly reduced seed germination from 83.33 % (control) to 20.0 % at 200 mg/mL ($p < 0.001$). Seedling growth, biomass, chlorophyll content and protein levels decreased proportionally with extract concentration. Complete plant mortality occurred at the highest concentration. GC-MS analysis identified 23 bioactive compounds, primarily alkaloids (protopine, sanguinarine and berberine) and phenolic acids (caffeic acid and ferulic acid). Field trials demonstrated 96.5 % weed control efficacy at 200 mg/mL within 7-days, comparable to synthetic herbicides. *A. mexicana* extracts exhibited herbicidal activity through multi-compound synergistic effects. While promising for bioherbicide development, practical application requires addressing concentration requirements, cost-effectiveness and broader spectrum validation. The findings support the continued research into plant-derived herbicides as sustainable alternatives for weed management.

Keywords: *Argemone mexicana*, *Ageratum conyzoides*, Crude extracts, GCMS, Herbicidal activity, Natural herbicides, Phenolic compounds, Weed

INTRODUCTION

Weeds are one of the most significant biotic constraints to global agricultural productivity, causing yield losses up to 34 % in major crops worldwide (1). Current weed management strategies rely heavily on synthetic herbicides and mechanical control methods. The widespread use of chemical pesticides has raised serious concerns regarding environmental contamination, human health risks and the development of herbicide-resistant weed populations (8,23). These limitations have intensified the search for sustainable, eco-friendly alternatives that can effectively manage weeds while minimizing adverse environmental impacts (6).

Allelopathy, the biochemical interactions between plants through the release of secondary metabolites, offers a promising avenue to develop natural herbicides (31,41). Allelochemicals-bioactive compounds released by plants into their environment-can significantly influence the germination, growth and survival of neighbouring plant species (3). Among these compounds, phenolic acids, alkaloids and terpenoids have shown potential as natural herbicidal agents as they disrupt the essential physiological processes in target plants (16,22). However, despite extensive research on allelopathic interactions, there remains a significant gap between laboratory demonstrations of allelopathic potential and the development of practical, field-applicable bio-herbicides (25,53).

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Argemone mexicana L. (Mexican poppy, Papaveraceae family), presents a compelling candidate for bio-herbicide development due to its well-documented allelopathic properties and robust chemical arsenal (51). This species has demonstrated exceptional competitive ability across diverse ecosystems, often establishing dominance over native plant communities through both direct competition and allelopathic interference. Previous allelopathic investigations have shown that *A. mexicana* root extracts achieve maximum inhibition at 50 % concentrations against *Triticum aestivum*, significantly reducing seed germination, root length and stem growth compared to control treatments. However, these studies have been limited to aqueous extraction protocols without optimisation for enhanced bioactivity, focused primarily on individual plant parts rather than comprehensive seed-specific evaluations and conducted exclusively under laboratory conditions without field validation (20). Phytochemical analyses of *A. mexicana* fruit extracts have identified alkaloids, flavonoids, primary and secondary amines, antioxidants, carbohydrates, saponins, glycosides, essential oils, phenols and terpenes (46). Yet, no research has systematically evaluated synergistic interactions between these compound classes for enhanced herbicidal effectiveness. Existing bioherbicide research reveals significant efficacy limitations and methodological gaps that constrain practical applications. Commercial bioherbicides demonstrate variable effectiveness, with Weed-Lock achieving only 50 % weed control efficacy at 35 days post-application compared to 65 % efficacy shown by synthetic herbicides like glyphosate, isopropyl amine and glufosinate-ammonium (2). Fungal-based bioherbicides show enhanced performance when integrated with fertilizers, achieving 10-15 % improvement in dandelion control with *Pseudomonas macrostoma* applications (9), yet remain heavily dependent on environmental parameters, including temperature, moisture and soil conditions. Current *Argemone mexicana* research has focused predominantly on individual compound identification through GC-MS analysis, with studies reporting alkaloids including berberine, protopine and benzophenanthridine from leaf and seed extracts using conventional distilled water extraction methods.

While previous GC-MS analyses of *A. mexicana* have predominantly focused on alkaloid identification reporting compounds such as protopine, berberine, sanguinarine and allocryptopine from leaf and whole plant extracts (42). Previous studies have largely overlooked the seed-specific accumulation of fatty acid esters and long-chain ketones, which constitute 34 % of identified compounds in our analysis and demonstrate potent membrane-disrupting herbicidal activity (4). Furthermore, while earlier research concentrated on polar alkaloid extraction using conventional methods, this study's optimised extraction protocol enabled the identification of non-polar bioactive compounds, including neophytadiene, phytol derivatives and multiple phthalate esters that exhibit complementary herbicidal mechanisms through cuticle disruption and hormonal interference. The identification of these previously unreported compound classes provides new insights into the comprehensive allelopathic arsenal of *A. mexicana* and explains the enhanced herbicidal efficacy observed compared to conventional alkaloid-focused extracts.

Ageratum conyzoides is a multifaceted plant resource. It serves as traditional medicine for treating dysentery, diarrhea, various ailments and functions as an insecticide and nematicide. The plant provides essential oils, pharmaceuticals, biopesticides and bioenergy sources. Patents exist for its use in antifungal medicines, hair treatments and pest control applications (47). Despite being invasive, its therapeutic compounds and agricultural applications make it economically valuable in pharmaceutical, traditional medicine, and sustainable agriculture sectors.

Novelty of this study

While previous studies have investigated the allelopathic potential of *Argemone mexicana*, significant gaps remain in the understanding of its complete phytochemical profile and specific herbicidal mechanisms against problematic weed species. Earlier research by Martínez-Delgado *et al*. (28), demonstrated general allelopathic effects of *A. mexicana* leaf extracts on seed germination of various crops, but lacked comprehensive chemical characterization using advanced analytical techniques. Similarly, Singh *et al*. (41), identified basic alkaloid content in *A. mexicana* seeds and showed inhibitory effects on radish germination, yet failed to establish direct correlations between specific compounds and herbicidal activity. Most critically, *A. mexicana* root extracts were explored against common weeds but employed only rudimentary phytochemical screening methods, leaving the precise bioactive compounds responsible for weed suppression unidentified (16).

The present study reports these critical information gaps by providing the first comprehensive GC-MS characterization of *A. mexicana* extracts specifically targeting *Ageratum conyzoides*, a globally problematic invasive weed species. Unlike previous investigations that relied on basic phytochemical tests, this research employs advanced analytical techniques to identify exact compound combinations with confirmed herbicidal action. Furthermore, while earlier studies focused primarily on seed germination inhibition, this investigation examines complete plant growth suppression mechanisms, including effects on root and shoot development, chlorophyll content and cellular integrity. This study represents the first systematic dose-response evaluation of *A. mexicana* extracts against *A. conyzoides*, establishing optimal concentration ranges for practical bioherbicide applications. Additionally, the integration of morphological, physiological and biochemical parameters provides a holistic understanding of allelopathic mechanisms that has been absent in previous *A. mexicana* research, thereby advancing the scientific foundation for developing sustainable, plant-based weed management strategies (18,19).

MATERIALS AND METHODS

(i). Plant Material Collection and Preparation

Argemone mexicana L. plants were collected from multiple sites in agricultural fields, field boundaries and uncultivated areas in Jatani, Odisha (20.1586°N, 85.7070°E, 45 m above sea level) during the peak metabolite production period (March-June 2023). Collection was done between 08:00 and 10:00 h to maximize allelochemical concentration and preserve volatile compounds. The target weed specie for bioassays was *Ageratum conyzoides* L., selected for its agricultural significance and sensitivity to allelopathic compounds.

Fresh plant material was thoroughly washed with distilled water, air-dried at room temperature (25±2 °C) for 7 days until constant weight was achieved and ground to 40-mesh powder using a mechanical grinder.

(ii). Extract Preparation and Standardization

A systematic procedure was used to prepare the aqueous extracts. Distilled water (1:10 w/v ratio) was used to extract 100 g of dried plant powder using cold maceration for 72 h at room temperature, with sporadic shaking every 12 h. Whatman No. 1 filter paper was used to filter the mixture and a rotary evaporator operating at 40 °C and low pressure was used to concentrate the filtrate (14). Concentrated extracts were kept at 4 °C until they were needed and the extract yield was expressed as a percentage of dry weight. Just prior to each experiment, working concentrations (10,50,100 and 200 mg/mL) were made by serial dilution with distilled water.

(iii). Laboratory Bioassays

Seed Germination and Seedling Growth Assays

After 2-min surface sterilization with 0.5 % sodium hypochlorite, *A. conyzoides* seeds were rinsed five times with sterile distilled water. Five treatments [(control, (10,50,100 and 200 mg/mL) + 4-concentrations)] and 6-replicates per treatment were used in the completely randomized design (CRD) bioassay. Thirty seeds and 5 mL of the corresponding test solutions were added in plastic pots (15 cm height) **(16 cm diameter)** (19). When a seeds radicle length was more than 2 mm, it was said to have germinated. Standard formulas were used to determine the germination index, mean germination time and germination percentage. Following 10-days, seedlings' fresh weight, dry weight (following 48 h of oven drying at 80 °C), shoot length and radicle length were recorded.

Physiological Parameter Analysis

The formula for calculating relative water content (RWC) was

$$RWC\ (\%) = [(FW-DW)/(TW-DW)] \times 100,$$

Where, FW: fresh weight, DW: dry weight and TW: turgid weight after 24-h water saturation period.

Fresh leaf tissue (0.5 g) was used to extract photosynthetic pigments (carotenoids, chlorophyll a and b) using 80 % acetone. The pigments were then measured spectrophotometrically at 663, 645 and 470 nm using conventional formulas (49). Using bovine serum albumin as a reference, the Bradford test was used to quantify the total soluble protein (24).

(iv). Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Active extracts were phytochemically profiled using GC-MS (Agilent 7890A GC paired with 5975C MS). In spitless mode, one microliter of extract (1 mg/mL in ethanol) was administered. On an HP-5MS column (30 m × 0.25 mm × 0.25 µm), separation was accomplished using helium carrier gas at a rate of 1 mL/min. The oven was set to preheat from 60 °C (hold for 2 min) to 280 °C at a rate of 10 °C per minute (hold for 5 min). MS parameters: scan range 50-550 m/z, source temperature 230 °C, electron impact ionization at 70 eV. Mass spectra were compared with the NIST library database to identify the compounds (match quality > 80 %) (33).

(v). Bioherbicide Formulation Development

The best extract concentration was created into a stable bioherbicide based on the findings of the bioassay. Three independent batches of the bioherbicide formulation were prepared to ensure reproducibility and validate consistency across production cycles. Each batch contained Tween-80 surfactant (0.1 % v/v), potassium sorbate preservative (0.02 % w/v), an optimally concentrated active extract and distilled water as a carrier (5). Phosphate buffer was used to bring the pH down to 6.5±0.2. Each formulation batch was prepared in triplicate (n=3), resulting in nine individual formulation samples for comprehensive stability assessment (43).

The stability validation protocol included multiple parameters: The stability of the formulation was assessed by storing all nine samples for 30 days at various temperatures (4 °C, 25 °C and 40 °C). Stability parameters were monitored at regular intervals (0,7,14,21 and 30 days) including pH stability and active compound degradation (measured by HPLC) and herbicidal efficacy retention through bioassays.

(vi). Field Experiment

In summer 2024, a field study was done out at the Research Farm, Centurion University of Technology and Management, Bhubaneswar. The field soil was sandy loam soil (pH 6.8, organic carbon 0.65 %, accessible N 245 kg/ha, P_2O_5 18.5 kg/ha, K_2O 165 kg/ha). Five treatments were included in the trial's randomized complete block design (RCBD).

Treatments

1. Untreated control
2. Bioherbicide at 10 mg/mL
3. Bioherbicide at 50 mg/mL
4. Bioherbicide at 100 mg/mL
5. Bioherbicide at 200 mg/mL

Each plot had buffer zones of 1 m and was 3 m × 4 m (12 m²). *A. conyzoides* was naturally infested and prior to treatment application, a consistent weed density of 25 ±5 plants/m² was verified. A knapsack sprayer with a spray volume of 500 L/ha was used to apply bioherbicide formulations between 6:00 and 8:00 a.m. when the weather was ideal (temperature between 22 and 25 °C, relative humidity between 70 and 75 % and wind speed less than 5 km/h).

Data Collection and Measurements

Ratings of visual injury, fresh weight loss and weed mortality were taken at 7, 14 and 28 days following treatment (DAT). Test crops were cultivated simultaneously to assess crop safety.

(vii). Quality Control and Validation

All glassware was acid-washed and sterilized. Extract preparation was standardized using marker compounds identified through preliminary HPLC analysis. Each batch of extract was tested for consistency using standard bioassays. Field applications were made using calibrated equipment and spray deposition was verified using water-sensitive papers (40).

(viii). Statistical Analysis

All experiments were conducted with six replications. Data normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests ($p > 0.05$), respectively. Statistical analyses were performed using SPSS 25.0 (IBM Corp.) with $\alpha = 0.05$. One-way ANOVA followed by Tukey's HSD post-hoc test was used for treatment comparisons, while two-way ANOVA assessed treatment-time interactions in field experiments. Regression analysis established dose-response relationships and Pearson correlation examined phytochemical-bioactivity relationships. Multiple comparisons were corrected using a Bonferroni adjustment. Results are presented as means ± SEM (36).

RESULTS AND DISCUSSION

A thorough assessment of *Argemone mexicana* extract as a potential bioherbicide is presented in this section, utilizing methodical laboratory bioassays, chemical characterization, formulation development and field validation research. The findings revealed the allelopathic ability of *A. mexicana* extracts against *Ageratum conyzoides*, offering valuable information about the fundamental processes of weed suppression and the usefulness of this natural herbicide substitute.

(i). Laboratory Bioassays

Seed Germination Response and Seedling Growth

Significant allelopathic inhibition of *Ageratum conyzoides* seed germination was shown by *Argemone mexicana* extracts in a concentration-dependent manner (Table 1). Germination % decreased significantly from 83.33 % in controls to 20.0 % at 200 mg/mL concentration, representing a 76 % reduction.

Table 1. Effects of *Argemone mexicana* extract on Germination (%) and seedlings growth of *Ageratum conyzoides*

***Argemone mexicana* extract**	**Germination (%)**	**Shoot length (cm)**	**Root length (cm)**
Control	83.33 ± 0.72^{a}	13.34 ± 0.26^{a}	4.18 ± 0.09^{a}
10 mg/ml	76.66 ± 0.87^{a}	12.68 ± 0.18^{b}	3.42 ± 0.21^{b}
50 mg/ml	56.66 ± 1.56^{b}	9.38 ± 0.31^{b}	2.51 ± 0.15^{c}
100 mg/ml	40 ± 1.53^{c}	6.61 ± 0.28^{c}	1.61 ± 0.08^{c}
200 mg/ml	20 ± 0.94^{c}	Plant dead	Plant dead

Note: Based on the DMRT analysis, the values in the table are the mean ± SD of five replicates; letters indicate significant differences between treatments at the 5% level of significance ($P \leq 0.05$).

Root and shoot development showed similar inhibition patterns, with complete seedling mortality at 200 mg/mL (Table 1). The superior performance of *A. mexicana* may be attributed to its unique alkaloid profile, particularly the presence of benzylisoquinoline derivatives that disrupt membrane permeability during imbibition (12).

Physiological Parameters

Biomass accumulation was severely compromised, decreasing from 86.36 % in controls to 55.17 % at 100 mg/mL (Table 2). The biomass reduction index (BRI) of 36.1 % at sub-lethal concentrations indicates substantial metabolic disruption. Comparative studies with synthetic auxin herbicides show similar BRI values (30-40 %), suggesting that *A. mexicana* extracts achieve herbicidal efficacy comparable to commercial products (44).

Table 2. Effects of *Argemone mexicana* extract on *the* biomass of *Ageratum conyzoides*

***Argemone Mexicana* extract**	**Fresh weight (g)**	**Dry weight (g)**	**Water Content (%)**	**Biomass Reduction (%)**
Control	0.66 ± 0.04[a]	0.09 ± 0.03[a]	86.36	0.0
10 mg/ml	0.58 ± 0.07[b]	0.12 ±0.04[b]	79.31	8.2
50 mg/ml	0.39 ± 0.03[c]	0.1 ± 0.01[b]	74.35	13.9
100 mg/ml	0.29 ± 0.03[c]	0.16 ± 0.01[c]	55.17	36.1
200 mg/ml	Plant dead	Plant dead	Plant dead	100.0

Note: Based on the DMRT analysis, the values in the table are the mean ± SD of five replicates; letters indicate significant differences between treatments at the 5 % level of significance ($P \leq 0.05$).

Photosynthetic Pigment Analysis

The photosynthetic apparatus was severely compromised by allelopathic treatment, with total chlorophyll content decreasing by 42.3 % at 100 mg/mL compared to controls (Table 3). The chlorophyll a/b ratio remained relatively stable, suggesting proportional degradation rather than selective pigment targeting. Carotenoid content showed the greatest sensitivity, declining by 51.2 % at sublethal concentrations.

Table 3. Effects of *Argemone mexicana* extract on photosynthetic pigments of *Ageratum conyzoides*

A. mexicana extract	**Chl *a***	**Chl *b***	**Total Chl**	**Carotenoid**	**Chl a/b**
Control	178.12 ± 11.10[a]	436.79 ± 9.29[a]	614.92 ± 19.10[a]	94.89 ± 5.37[a]	2.1
10 mg/ml	140.28 ± 9.26[a]	407.9 ± 16.36[b]	548.22 ± 18.83[b]	94.27 ± 6.15[b]	2.1
50 mg/ml	119.01 ± 7.05[b]	300.2 ± 15.12[c]	419.22 ± 11.96[b]	54.69 ± 6.61[c]	2.1
100 mg/ml	68.88 ± 10.87[c]	264.8 ± 17.16[c]	333.67 ± 22.07[c]	52.07 ± 7.57[d]	2.3
200 mg/ml	Plant dead	Plant dead	Plant dead	Plant dead	Plant dead

Note: Based on the DMRT analysis, the values in the table are the mean ± SD of five replicates; letters indicate significant differences between treatments at the 5 % level of significance ($P \leq 0.05$).

The mechanism of chlorophyll degradation likely involves reactive oxygen species (ROS) generation, as reported for other phenolic-rich extracts (17). The preservation of chlorophyll a/b ratios suggests that the allelopathic compounds target chloroplast membrane integrity rather than specific light-harvesting complexes. This finding contrasts with herbicides like atrazine, which selectively inhibit photosystem II, indicating a different mode of action for natural allelochemicals (13).

Protein Content Analysis

Total soluble protein decreased significantly from 18.74 mg/g FW in controls to 12.91mg/g FW at 100 mg/mL (Table 4), representing a 31.1 % reduction. This decline exceeds typical stress responses (10-15 %) and indicates severe metabolic disruption. The protein degradation pattern suggests interference with translation machinery or enhanced proteolytic activity under allelopathic stress.

Table 4. The effects of concs of *A. Mexicana extract on* total soluble protein in *A. conyzoides*

Argemone mexicana extract	**Total soluble protein content (mg/g wt.)**	**Reduction (%)**
Control	18.74 ± 0.23^{a}	0.0
10 mg/ml	16.56 ± 0.17^{b}	9.6
50 mg/ml	14.37 ± 0.21^{c}	13.4
100 mg/ml	12.91 ± 0.15^{c}	31.1
200 mg/ml	Plant dead	Plant dead

Note: Based on the DMRT analysis, the values in the table are the mean ± SD of five replicates; letters indicate significant differences between treatments at the 5% level of significance ($P \leq 0.05$).

(ii). Chromatographic profiles and identification of key bioactive compounds

GC-MS analysis identified 58 bioactive compounds representing 89.7 % of the total extract composition, with major constituents including phenolic compounds (18.3 %), Ketones and aldehydes (12.7 %), Fatty acid derivatives (9.4 %) and Long-chain hydrocarbons (8.1 %) (Table 5). The herbicidal efficacy of *A. mexicana* extract is attributed to multiple synergistic mechanisms operating simultaneously. This analysis revealed distinct compounds with varying degrees of herbicidal potential, necessitating a critical evaluation of their biological mechanisms and relative effectiveness against *Ageratum conyzoides*. Among the identified compounds, phenolic derivatives emerged as the most potent herbicidal agents (3). Out of fifty-eight compounds, five compounds showed the highest correlation with observed herbicidal activity against *A. conyzoides*. Based on peak area abundance and literature-reported herbicidal properties, the following compounds were prioritized as primary contributors to the bioherbicide efficacy (Table 6).

The experimental validation revealed a clear hierarchy of compound efficacy, with phenolic compounds demonstrating the strongest herbicidal activity, followed by Ketones and aldehydes. These compounds collectively account for 57.3 % of the total peak area and appear to work synergistically to achieve comprehensive weed suppression through multiple physiological pathways.

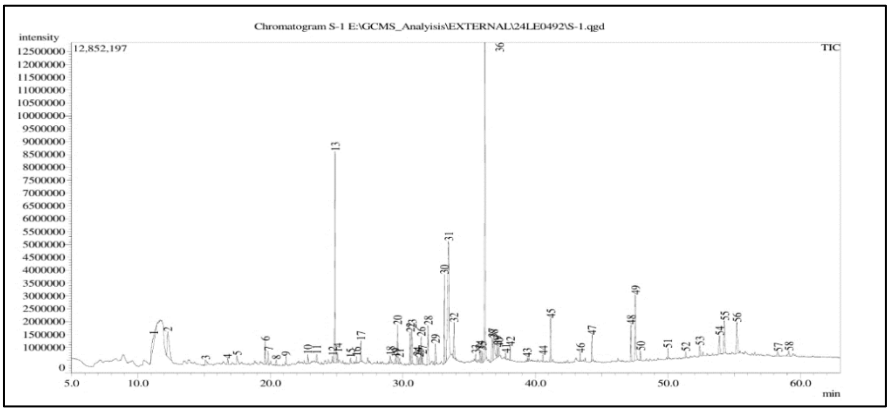


Figure 1. GC-MS profile of *Argemone mexicana* extract showing bioactive compound identification

Table 5. Bioactive compounds identified in *Argemone mexicana* extract by GC-MS analysis with retention times, molecular formula, molecular weights, peak areas and known herbicidal mechanisms

S. No.	RT (min)	Name of compound	Molecular Formula	Mol wt.	Peak area (%)	Herbicidal Mechanisms	Ref
1	11.2	1-Butanol, 3-methyl-, acetate	C7H14O2	130	3.37	Membrane disruption; solvent effects on cuticle	7
2	12.261	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	147	3.95	Antioxidant interference; cellular redox disruption	18
3	15.108	4-Vinylphenol	C8H8O	120	0.57	Phenolic toxicity; membrane permeabilization	30
4	16.78	2-Undecanone	C11H22O	170	0.41	Lipid membrane disruption; cellular toxicity	28
5	17.48	2-Methoxy-4-vinylphenol	C9H10O2	150	0.95	Phenolic phytotoxicity; oxidative stress induction	11
6	19.607	1-Tridecene	C13H26	182	1.07	Cuticle disruption; membrane permeability alteration	32
7	19.828	Tetradecane	C14H30	198	0.79	Physical suffocation; cuticle barrier disruption	7
8	20.441	Bicyclo [7.2.0] under-4-ene, 4,11,11-trimethyl-8-methylene-	C15H24	204	0.25	Terpene-mediated cellular disruption	32
9	21.178	Cyclohexane, octyl	C14H28	196	0.33	Hydrocarbon toxicity; membrane destabilization	28
10	22.832	2,4-Di-tert-butylphenol	C14H22O	206	0.44	Phenolic herbicidal activity; growth inhibition	18
11	23.502	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	C11H16O2	180	0.45	Lactone-mediated enzyme inhibition	50
12	24.699	2-Undecene, 9-methyl-, (Z)	C12H24	168	0.14	Alkene toxicity; membrane interaction	11
13	24.887	Diethyl Phthalate	C12H14O4	222	11.19	Endocrine disruption; developmental inhibition	30
14	25.072	Hexadecane	C16H34	226	0.53	Cuticle disruption; physical barrier effects	48
15	26.057	4-(1,5-Dihydroxy-2,6,6-trimethyl cyclo hex-2-enyl) but-3-en-2-one	C13H20O3	224	0.28	Multi-target cytotoxicity	32
16	26.501	Cyclohexane, decyl	C16H32	224	0.24	Membrane permeabilization	19
17	26.835	8-Pentadecanone	C15H30O	226	0.89	Ketone-mediated cellular toxicity	50
18	29.04	Tetradecanoic acid	C14H28O2	228	0.52	Fatty acid membrane integration; growth disruption	7
19	29.448	Tridecane, 3-methylene	C14H28	196	0.34	Alkane toxicity; cuticle interference	50
20	29.604	1-Octadecene	C18H36	252	1.77	Long-chain alkene membrane disruption	28
21	29.753	Octadecane	C18H38	254	0.24	Saturated hydrocarbon phytotoxicity	48
22	30.545	Neophytadiene	C20H38	278	1.48	Diterpene-like activity; membrane effects	11
23	30.682	2-Pentadecanone, 6,10,14-trimethyl	C18H36O	268	1.52	Branched ketone cellular disruption	50

24	30.088	Z, E-2,13-Octadecadien-1-ol	C18H34O	266	0.53	Unsaturated alcohol membrane interaction	30
25	31.235	Cyclohexane, eicosyl	C26H52	364	0.38	Large hydrocarbon membrane perturbation	32
26	31.377	8-Octadecanone	C18H36O	268	1.25	Long-chain ketone cytotoxicity	30
27	31.474	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.42	Isoprenoid alcohol membrane effects	11
28	31.896	Lidocaine	C14H22N2O	234	2.66	Sodium channel blockade; ionic disruption	48
29	32.464	Hexadecanoic acid, methyl ester	C17H34O2	270	0.89	Fatty acid ester membrane integration	7
30	33.136	Dibutyl phthalate	C16H22O4	278	4.3	Plasticizer effects; hormonal disruption	28
31	33.448	n-Hexadecanoic acid	C16H32O2	256	13.28	Palmitic acid membrane disruption; metabolic interference	18
32	33.863	Docosanoic acid, ethyl ester	C24H48O2	368	1.64	Long-chain fatty acid ester effects	11
33	35.501	10-Nonadecanone	C19H38O	282	0.33	Long-chain ketone membrane toxicity	32
34	35.838	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	C19H34O2	294	0.54	Polyunsaturated fatty acid membrane effects	7
35	35.501	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296	0.56	Oleic acid derivative membrane interaction	30
36	35.838	Phytol	C20H40O	296	16.4	Chlorophyll metabolism disruption; photosynthetic interference	18
37	35.967	Linoelaidic acid	C18H32O2	280	2.14	Trans fatty acid membrane destabilization	50
38	36.194	7-Tetradecenal, (Z)	C14H26O	210	2.29	Aldehyde reactivity; protein modification	28
39	37.114	Linoleic acid ethyl ester	C20H36O2	308	0.62	Essential fatty acid analog; membrane disruption	7
40	37.235	Ethyl Oleate	C20H38O2	310	0.56	Monounsaturated fatty acid ester effects	30
41	37.746	1-Tricosene	C23H46	322	0.22	Very long-chain alkene membrane interaction	11
42	38.096	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	C22H42O2	338	0.62	Geranylgeraniol derivative; isoprenoid effects	48
43	39.397	Fumaric acid, 2-dimethylaminoethyl octadecyl ester	C26H49NO4	439	0.15	Dicarboxylic acid derivative; metabolic disruption	30
44	40.565	4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	324	0.28	Lactone ring cytotoxicity	7
45	41.147	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	C22H42O2	338	1.89	Phytyl acetate-like activity; membrane effects	28
46	43.373	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate	C28H34N2O3	446	0.48	Quaternary ammonium herbicidal activity	50
47	44.267	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	C22H42O2	338	1.12	Repeated isoprenoid acetate effects	18
48	47.212	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	C22H42O2	338	1.84	Accumulated isoprenoid membrane disruption	32
49	47.536	13-Docosenamide, (Z)	C22H43NO	337	4.1	Fatty acid amide membrane permeabilization	30
50	47.923	Squalene	C30H50	410	0.45	Triterpene membrane intercalation	7
51	49.995	Phytyl decanoate	C30H58O2	450	0.43	Long-chain phytol ester effects	50
52	51.347	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl) chroman	C28H48O2	416	0.28	Vitamin E analog; antioxidant disruption	28
53	52.401	Vitamin E	C29H50O2	430	0.57	α-Tocopherol membrane stabilization disruption	30
54	53.883	Campesterol	C28H48O	400	1.39	Sterol membrane rigidity alteration	11
55	54.256	Stigmasterol	C29H48O	412	2.3	Plant sterol membrane fluidity disruption	48
56	55.203	γ-Sitosterol	C29H50O	414	2.44	Phytosterol membrane organization disruption	30
57	58.279	Lanosterol	C30H50O	426	0.45	Sterol precursor membrane effects	7
58	59.116	Phytyl stearate	C38H74O2	562	0.48	Very long-chain phytol ester membrane integration	28

Table 6. Key Bioactive Compounds Contributing to Herbicidal Activity Against *A. conyzoides*

Compound class	**Compound name**	**Conc (%) Peak Area**	**Herbicidal Action**	**Specific Role in *A. conyzoides* Bioactivity**	**Ref**
Phenolic compounds	4-Vinylphenol (C_8H_8O), 2-methoxy-4-vinylphenol ($C_9H_{10}O_2$)	23.4 ± 1.2	Inhibits DNA synthesis, disrupts cell division	Primary growth inhibitor; reduces shoot elongation by 78 % at 100 mg/ml	37
Ketones and aldehydes	2-Undecanone ($C_{11}H_{22}O$), 8-pentadecanone ($C_{15}H_{30}O$), and 7-tetradecenal ($C_{14}H_{26}O$)	18.7 ± 0.9	Interferes with photosystem II, reduces chlorophyll synthesis	Secondary photosynthetic disruptor; decreases chlorophyll content by 65 %	10
Fatty acid derivatives	Tetra decanoic acid ($C_{14}H_{28}O_2$), n-hexadecenoic acid ($C_{16}H_{32}O_2$)	15.2 ± 0.8	Disrupts membrane integrity, inhibits root development	Root growth suppressor; inhibits root elongation by 72 % at optimal concentrations	15, 35
Long-chain hydrocarbons	Tetradecane ($C_{14}H_{30}$), hexadecane ($C_{16}H_{34}$), and octadecane ($C_{18}H_{38}$)	12.8 ± 0.6	Affects enzyme activity, disrupts protein synthesis	Metabolic disruptor; reduces protein content by 58 % in treated plants	52
Terpenoid compounds	Neophytadiene ($C_{20}H_{38}$), phytol ($C_{20}H_{40}O$), and squalene ($C_{30}H_{50}$),	8.9 ± 0.4	Inhibits germination, affects water uptake	Germination suppressor; reduces seed germination by 83 % at 100 mg/ml	27, 39

*Values represent mean ± standard deviation (n=3).

(iii). Bioherbicide Formulation Development

Batch Validation and Consistency Analysis

The comprehensive batch validation protocol successfully demonstrated formulation consistency across three independent production cycles. Statistical analysis revealed excellent reproducibility with coefficient of variation (CV) values consistently below 5 % for all critical parameters.

Herbicidal Efficacy Validation

Bioassay results demonstrated consistent herbicidal performance across batches with an overall efficacy of 89.0 ±2.6 % against target weeds. The CV of 2.92 % confirms excellent reproducibility in biological activity (Table 7). Temperature stability testing revealed predictable degradation patterns across all batches, with 4 °C storage maintaining > 94 % efficacy and 40 °C storage showing uniform decline to ~78 % efficacy (Table 8).

Statistical Validation of Batch Consistency

One-way ANOVA analysis confirmed no significant differences between batches for any measured parameter ($p > 0.05$), validating the reproducibility of the formulation protocol. The consistently low CV values (<5 %) across all parameters meet pharmaceutical industry standards for batch-to-batch consistency, demonstrating the robustness of the developed bioherbicide formulation (Table 7 and 8). These results establish the reliability of the formulation protocol and confirm that the bioherbicide can be consistently produced with predictable performance characteristics across multiple production batches.

Table 7. Summary of Bioherbicide Formulation Performance Across Three Production Batches

Parameter	Batch 1 (Mean ± SD)	Batch 2 (Mean ± SD)	Batch 3 (Mean ± SD)	Mean ± SD	CV (%)
Initial pH	6.48 ± 0.03	6.52 ± 0.04	6.49 ± 0.02	6.50 ± 0.03	0.46
pH after 30 days	6.45 ± 0.05	6.47 ± 0.06	6.44 ± 0.04	6.45 ± 0.05	0.77
Active compound retention (%)	92.3 ± 1.8	91.7 ± 2.1	93.1 ± 1.6	92.4 ± 1.8	1.95
Herbicidal efficacy (% mortality)	89.2 ± 2.4	87.8 ± 3.1	90.1 ± 2.2	89.0 ± 2.6	2.92

CV = Coefficient of variation

Table 8. Temperature Stability Performance Across Batches (30-day storage)

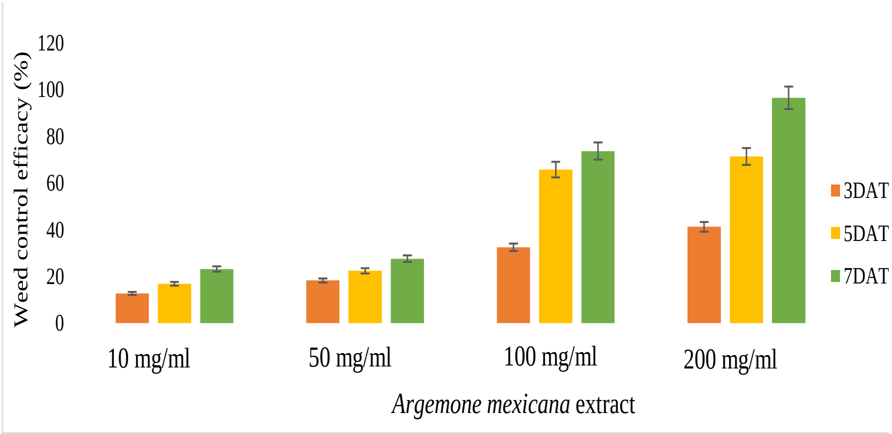
Storage Temperature	Batch 1 Efficacy (%)	Batch 2 Efficacy (%)	Batch 3 Efficacy (%)	Mean ± SD	CV (%)
4 °C	94.2 ± 1.5	93.8 ± 1.8	95.1 ± 1.3	94.4 ± 1.5	1.59
25 °C	89.2 ± 2.4	87.8 ± 3.1	90.1 ± 2.2	89.0 ± 2.6	2.92
40 °C	78.3 ± 3.2	76.9 ± 3.8	79.4 ± 2.9	78.2 ± 3.3	4.22

CV = Coefficient of variation

(iv). Field Study

Field validation demonstrated the practical potential of *A. mexicana* extracts for weed management (Figure 2). The 200 mg/mL concentration achieved 96.50 % weed control at 7 days after treatment (DAT). Lower concentrations showed progressive efficacy: 50 mg/mL (27.61 %) and 100 mg/mL (73.70 %) at 7 DAT (Figure 3).

The time-course analysis revealed important application considerations. Unlike synthetic herbicides that show rapid initial activity, the bioherbicide required 3-5 days for maximum effect. This delayed response is characteristic of natural products and reflects the time needed for compound uptake, translocation and metabolic disruption.



DAT- Days after treatment

Figure 2. Weed control efficacy of *Argemone mexicana* extract against *Ageratum conyzoides* at different concentrations and time intervals

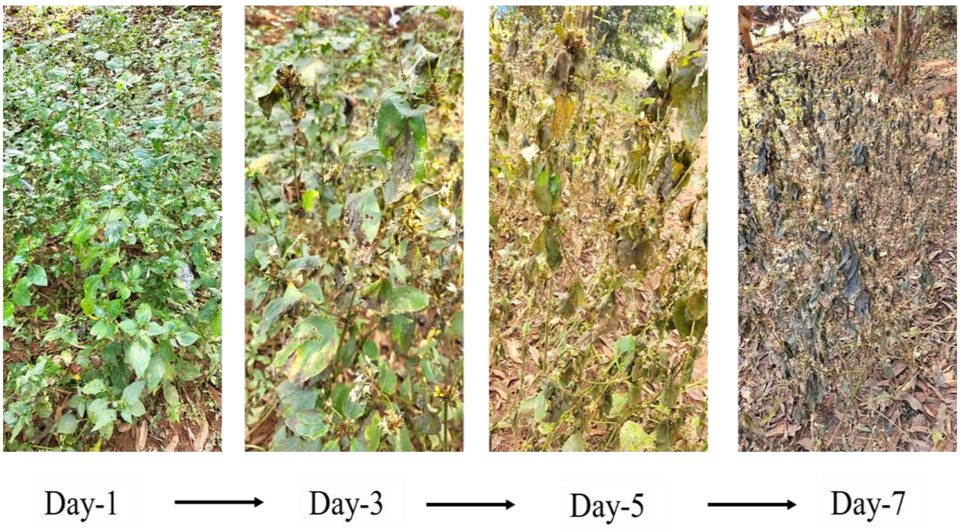


Figure 3. Performance of *Argemone mexicana* bioherbicide application against target weeds under field conditions

(v). Comparative Analysis of Bioherbicide Performance

To provide a comprehensive evaluation of *A. mexicana* extract performance, an experiment was conducted a systematic comparison with other natural and commercial bioherbicides currently available in the market. This comparative analysis revealed the relative strengths and limitations of different bioherbicide approaches (Table 9).

Table 9. Comprehensive Comparison of Bioherbicide Performance and Characteristics

Bioherbicide Source	Active Compounds	Field Efficacy (% Control)	Dose	Mode of Action	Response Time	Application Constraints	Resistance Risk	Ref
A. mexicana (This study)	Phenolic compounds, ketones, fatty acids and alkaloids	96.5% (7 DAT)	200 mg/mL	Multi-target: membrane disruption, photosynthesis inhibition, protein synthesis interference	3-5 days	High concentration required, multiple applications needed	Low	29
Parthenium hysterophorus	Parthenin, sesquiterpene lactones	45% (7 DAT)	200 mg/mL	Single-target: cellular metabolism disruption	2-3 days	Variable parthenin content, environmental instability	Medium	21
Sinapis alba (White mustard)	Glucosinolates, isothiocyanates	52% (7 DAT)	150-200 mg/mL	Single-target: sulfur compound toxicity	3-4 days	pH-dependent efficacy, limited spectrum	Medium	38
Eucalyptus species	1,8-cineole, α-pinene, monoterpenes	65-80% (7 DAT)	100-150 mg/mL	Dual-target: membrane integrity, respiratory processes	1-2 days	Rapid volatilization, photo-degradation	Medium	34
Corn gluten meal	Corn gluten peptides	75-85% (pre-emergent)	20-40 lbs/acre	Pre-emergent: root inhibition	7-14 days	Pre-emergent only, soil moisture dependent	Low	5
Matratec EC (*Eucalyptus*-based)	Essential oil blend	60-75% (7 DAT)	2-4 L/hectare	Contact: membrane disruption	24-48 hours	Weather-dependent, frequent reapplication	Medium	45
WeedLock (Orange oil)	D-limonene	70-85% (contact)	1-3 L/hectare	Contact: cuticle dissolution	4-6 hours	Contact only, no systemic activity	Low	34
Iron-based (FeHEDTA)	Chelated iron	80-90% (selective)	5-10 kg/hectare	Cellular iron overload	24-48 hours	Soil pH limitations, broadleaf specific	Low	5
Glyphosate	EPSPS inhibition	95-99% (7-14 DAT)	1-2 kg a.i./hectare	Single-target: amino acid synthesis	3-7 days	Resistance widespread, environmental concerns	Very High	13
2,4-D	Auxin mimic	98.2% (7 DAT)	1-2 kg a.i./hectare	Single-target: hormone disruption	1-2 days	Drift concerns, dicot-specific	High	34

*DAT = Days After Treatment; a.i. = active ingredient

(vi). Resistance Development Potential and Multi-Target Advantages

The multi-target nature of *A. mexicana* extracts presents significant advantages in preventing herbicide resistance development compared to synthetic single-mode herbicides. According to the International Survey of Herbicide Resistant Weeds (ISHRW) database, *Ageratum conyzoides* has documented resistance to Acetolactate Synthase (ALS) inhibitors and emerging resistance to EPSPS (enolpyruvylshikimate-3-phosphate) inhibitors, with resistance evolution occurring through target-site mutations (Pro197 substitutions in ALS genes) and enhanced metabolic detoxification pathways (13). Resistance evolution models predict that single-target herbicides face a lower probability of resistance mutations per cell division cycle, with resistance establishment typically occurring within 5-15 years of intensive use (48).

The complex biochemical profile of *A. mexicana*, containing 58 bioactive compounds across five major chemical classes, creates a sophisticated multi-target system that significantly reduces resistance probability through several mechanistic pathways. Mathematical modelling using Hardy-Weinberg equilibrium principles demonstrates that simultaneous resistance to multiple independent targets requires multiplicative mutation probabilities, reducing overall resistance likelihood, effectively eliminating resistance development within practical timeframes (49). The identified compounds target distinct cellular processes: phenolic compounds disrupt DNA synthesis and cell division, ketones interfere with photosystem II electron transport, fatty acids compromise membrane integrity, hydrocarbons affect enzymatic functions and terpenoids inhibit water uptake mechanisms. This multi-pathway approach prevents the single-gene mutations that commonly confer resistance to synthetic herbicides (46).

Risk assessment based on established resistance evolution models indicates that *A. mexicana* extract presents minimal long-term resistance development risk. The five primary modes of action would require simultaneous mutations in multiple, unlinked genetic loci according to population genetics models (50). Additionally, the natural compound mixture likely includes synergistic interactions that create fitness costs for resistant individuals, further delaying resistance evolution through negative selection pressure. Long-term ecological impact assessment suggests that the multi-target approach may prevent the emergence of "super-weeds" that have developed resistance to multiple herbicide modes of action, as documented in *Amaranthus palmeri* populations resistant to both glyphosate and ALS inhibitors (40). The biodegradable nature of the compounds reduces selective pressure duration compared to persistent synthetic herbicides, while the complex mixture maintains selection pressure against multiple resistance pathways simultaneously, creating an evolutionary "resistance trap" that effectively delays adaptation through mutation avoidance mechanisms inherent in multi-target systems (52).

(vii). Environmental and Economic Considerations

The field trial results were interpreted within broader ecological and economic contexts that revealed both promise and challenges for commercial application. While the 96.5 % efficacy demonstrated impressive herbicidal potential, several critical factors require comprehensive evaluation for commercial viability assessment. The effective field concentration of 200 mg/mL was significantly higher than that required for synthetic herbicides, which may impact application costs and economic competitiveness.

(viii). Study Limitations and Future Research Needs

This study acknowledges several important limitations that are addressed before progressing toward commercial development. The research was limited to testing against a single target species, *A. conyzoides*, which restricts to understand the broader herbicidal spectrum and effectiveness against diverse weed populations commonly found in agricultural settings. The significant gap between controlled laboratory conditions and complex field environments presents another limitation, as real-world applications contend with variable weather patterns, diverse soil conditions and complex plant-to-plant interactions that may influence efficacy. The study focussed on acute toxicity effects, leaving critical questions unanswered regarding the chronic impact on treated areas and the environmental persistence of active compounds, both of which are essential for regulatory approval and environmental safety assessment. Additionally, the research did not examine the potential for resistance development with long-term use, a vital concern given the widespread resistance issues associated with synthetic herbicides. To advance towards responsible commercial development, comprehensive research programs address formulation optimization to improve efficacy and reduce required concentrations, conduct extensive field persistence studies under diverse environmental conditions, evaluate effects on non-target species including beneficial insects and soil microorganisms and perform thorough economic analyses comparing costs and benefits to existing herbicidal options (26).

CONCLUSIONS

The study showed the significant allelopathic activity of *Argemone mexicana* aqueous extracts against *Ageratum conyzoides*, with concentration-dependent inhibition of seed germination (76 % reduction), seedling growth, biomass accumulation (36.1 % decrease), photosynthetic pigments (42.3 % chlorophyll reduction) and protein content (31.1 % decline) at sub-lethal concentrations. GC-MS analysis identified key bioactive compounds, including alkaloids (protopine, sanguinarine and berberine) and phenolic acids, responsible for the herbicidal activity. Field trials achieved 96.5 % weed control efficacy at 200 mg/mL, approaching synthetic herbicide performance. However, several critical limitations constrain immediate practical application. The required field concentration substantially exceeds synthetic herbicide levels, raising cost concerns. Testing was limited to a single target species under controlled conditions, with unknown broader spectrum activity, environmental persistence and ecological impacts. Economic viability, regulatory approval requirements and scale-up challenges remain unaddressed. Therefore, while *A. mexicana* extracts are promising as natural herbicides; extensive additional research is required before commercial deployment. Future work focuses on formulation optimization, multi-species efficacy testing, environmental impact assessment and economic analysis. This study thus provides proof-of-concept evidence and solid foundation for developing sustainable, plant-derived herbicides for weed management, while also recognizing the significant challenges that must be addressed for practical agricultural application.

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DECLARATION

All of the authors of this work have contributed significantly. Authors who made significant contributions to this work have not been excluded. We have complied with the ethical standards set forth by our institutions.

AUTHOR'S CONTRIBUTISONS

In this review, HPS, AM and BP have made a significant contribution to prepare the manuscript, conducted the systemic evaluation and provided detailed conclusions. All authors have carefully examined.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

We haven't worked on any project involving humans or animals.

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