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RESEARCH ARTICLE

Phytochemical profiling and functional evaluation of botanical extracts for herbicidal and growth-promoting activities

Meryani M Lotha[1], Ponnusamy Janaki[1,2*], Ramanujam Krishnan[2], Mariappan Suganthy[2], Etiyagounder Parameswari[2], Vadivel Vinothkumar[2] & Muringatheri Ramaiyer Latha[3]

[1]Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[2]Nammazhvar Organic Farming Research centre, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[3]Department of Soil Science and Agricultural Chemistry, Office of the Controller of Examination, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Correspondence email - janaki.p@tnau.ac.in

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Abstract

Weed management remains a major challenge in organic and natural farming, where synthetic herbicides are avoided. In this study, extracts of three botanicals *Cymbopogon citratus* (CC), *Lantana camara* (LC) and *Mangifera indica* (MI) were prepared using cow urine and water to explore their potential as natural herbicides. Metabolomic and mineral analyses revealed distinct chemical profiles, with cow urine extraction enhancing the release of bioactive compounds and nutrients. Weed suppression efficiency (WSE) showed a clear dose-dependent response, with the cow urine extract of CC (CCCU) achieving the highest activity (up to 31.89 %), outperforming water-based extracts and other botanicals. The superior performance of CCCU was linked to its higher phenolic and flavonoid content. Although less potent than synthetic herbicides, these botanicals offer dual benefits of weed suppression and nutrient enrichment, highlighting their potential role in sustainable and eco-friendly farming practices.

Keywords: allelocompounds; cow urine; LC-MS/MS analysis; metabolomics; nutrients; weed management

Introduction

The growing concerns surrounding the environmental and health impacts of synthetic herbicides have intensified the search for eco-friendly and sustainable alternatives in modern agriculture. Among these, plant-based extracts and animal-derived bio-inputs such as cow urine have gained attention due to their natural origin, biodegradability and multifaceted biological activities. Botanical extracts are known to be rich in phytohormones, amino acids and secondary metabolites such as flavonoids, phenols, terpenoids and alkaloids, which are often responsible for allelopathic and biostimulant effects on plants (1).

Cow urine, a traditional input in organic farming systems, is a complex mixture of nutrients, enzymes and microbial communities. It contains essential macro- and micronutrients (2), along with secondary metabolites such as benzoic acid derivatives and fatty acid methyl esters (3), which have been associated with antimicrobial and phytotoxic effects. Although cow urine alone may have limited herbicidal strength, its combination with botanical extracts may lead to synergistic interactions that enhance phytotoxic efficacy against weeds. Previous reports suggest that such mixtures can improve the stability, solubility and systemic mobility of allelochemicals in plant systems (4).

Additionally, the presence of growth-promoting hormones like indole-3-acetic acid (IAA), gibberellic acid (GA) and kinetin in leaf extracts, especially from *Cymbopogon citratus*, underscores their potential as natural biostimulants. These compounds are involved in plant growth regulation, stress signalling and root development (5, 6). Amino acids such as proline, serine and glutamic acid, detected in *Lantana camara* and *C. citratus*, further contribute to osmoprotection and nitrogen metabolism (7, 8).

Despite this knowledge, most studies have either examined cow urine or botanical extracts in isolation, with limited insights into their combined effects on weed suppression and nutrient solubilization. Moreover, little is known about how different extraction media influence the chemical profiles and biological efficacy of these botanicals. Addressing this gap, the present study aimed to extract phytochemicals and minerals from the allelopathic leaves of lemon grass (*C. citratus* (DC.) Stapf.), lantana (*L. camara.* L.) and mango (*Mangifera indica* L.) using cow urine and water as extraction media. The extracts were characterized through metabolite profiling by LC-MS/MS and mineral composition analysis and their potential as natural herbicides and growth enhancers was systematically evaluated.

Materials and Methods

Collection and phytochemical profiling of botanicals and extracts

Leaves of *C. citratus*, *L. camara* (LC) and *M. indica* (MI) were collected from the orchard and Eastern block farm area of TNAU and dried in partial shade and then ground to powder. The cow urine was collected from the Jersey breed in dairy farm of Tamil Nadu Agricultural University (TNAU) and stored in airtight container and used within 5 days of collection for the extraction. The phytochemical profiles of cow urine and raw botanical powders were qualitatively assessed for the presence of alkaloids, saponins, terpenoids, steroids, tannins, flavonoids, phenols, glycosides and related compounds (9).

Organic extraction of botanicals and testing on weeds

The dried botanical leaf powder was soaked in cow urine or water at powder: solvent ratios of 1:4, 1:5, 1:6.7, 1:10, 1:20 and 1:50 (w:v), corresponding to 25 %, 20 %, 15 %, 10 %, 5 % and 2 % (w/v) stock extracts respectively. The percent stock extract was determined by the following formula:

Percent stock extract

$$= \text{mass of powder (g)} / \text{volume of solvent (mL)} \times 100$$

For each ratio, the powder and solvent were combined and soaked for 1, 5 and 10 days in sealed containers at room temperature with daily gentle agitation. After soaking, extracts were recovered by filtration through muslin cloth with hand compression; the filtrates were collected, labelled with their percent (w/v) and the final volumes were recorded. The obtained extracts were applied against broad-leaved (*Trianthema portulacastrum*) and grass (*Chloris barbata*) weeds grown in disposable containers under laboratory *in vitro* conditions. All masses and volumes were measured using an analytical balance and graduated cylinder to ensure reproducibility. For each treatment, three biological replicates were prepared. In the weed assay, 10 weeds were maintained per cup, with each treatment replicated three times. Throughout the study, glyphosate at 5 mL/L of water was used as the standard check, beside control (water alone). The experiment was conducted under controlled growth conditions at 35 ± 2 °C with a 16/8 hr light/dark photoperiod.

These weeds were chosen based on the dominance composition occur in the organic farming field of TNAU farm grown with sorghum crop. Each extract was applied as a post-emergence spray at the 2–3 leaf stage of weeds (15 days after sowing). Weed biomass from each treatment was collected on the 10th day after spraying, dried and weighed to calculate weed suppression efficiency (WSE) (10). Based on the cup study screening, extracts with higher WSE along with their respective fermentation periods and concentrations were selected for triple quadrupole liquid chromatography–tandem mass spectrometry (LC–MS/MS) and phytochemical profiling. Day 1 fermentation samples were chosen for MI and LC, while day 10 fermentation samples were selected for CC. The selected botanical extracts were evaluated for pH and electrical conductivity (EC). The raw CC extract recorded a pH of 5.37 with an EC of 3.77, whereas LC exhibited a pH of 7.51 and an EC of 1.70. In contrast, MI showed a pH of 6.70 with an EC of 3.77.

Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS)

The 25 % concentrated extracts were subjected to untargeted metabolite profiling, targeted amino acid and growth hormone analysis and mineral composition determination. For metabolite extraction, 15 mL of methanol was added to 5 mL of cow urine or botanical extract and the mixture was processed by separating and discarding the aqueous layer while collecting the organic layer. This procedure was repeated 3-4 times to ensure thorough extraction. The pooled organic extracts were concentrated using a rotary evaporator, reconstituted in 4 mL of methanol and filtered through a 0.2 µm nylon membrane filter. The resulting filtrates were transferred into LC-MS/MS vials. As the analysis was performed in untargeted mode for qualitative metabolite profiling, internal standards and quality control samples were not included, while instrument stability was ensured through blanks and retention time monitoring.

Non-targeted metabolite profiling was performed using a Shimadzu LC-MS 8040 triple quadrupole with electrospray ionization (ESI) source and triple quadrupole mass spectrometer. Separation was achieved on a C18 column (4.6 mm × 250 mm, 5 µm, TMS end-capped) at 35 °C, with a 10 µL injection volume and m/z range of 100-1000. The mobile phase consisted of 0.1 % formic acid in water (A) and methanol (B) under a gradient program (5 % B, 0 min-2 min; ramp to 90 % B, 10 min; back to 5 % B, 15 min; held until 20 min) at 0.2 mL/min. MS data acquisition was performed in both positive and negative ion modes (drying gas 17 L/min, nebulizing gas 3 L/min, total flow 0.7 µL/min).

Each treatment was replicated three times, with three independent extracts prepared and analysed per treatment. For metabolite identification, the compounds corresponding to the observed m/z values were cross-checked using the Plant Metabolome Database (PmDB), as this library was not integrated into the instrument software.

Mineral composition assessment

Elemental analysis of cow urine and botanical extracts was conducted after acid digestion (11). While, nitrogen (N) was determined by the Kjeldahl method, the phosphorus (P) and potassium (K) were measured by UV-Vis spectrophotometry (420 nm) and flame photometry respectively. The concentrations of secondary nutrients, micronutrients and heavy metals were determined using a Thermo Fisher 7000 Series ICP-OES (USA) instrument. Samples were digested with nitric acid and appropriately diluted prior to analysis. Calibration curves were prepared using certified multi-element standard solutions (1000 mg/L stock; Merck or equivalent), diluted to working concentrations of 0.1, 0.5, 1, 5 and 10 mg/L for macro and micronutrient determination. Standards included essential elements such as C, N, P, K, calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni) and lead (Pb) to match the analytes of interest. Quality control samples and blank runs were included at regular intervals to verify accuracy and precision throughout the analysis.

Statistical analysis

All data were collected in triplicate. Non-targeted metabolomic profiles were analysed using Metabo Analyst 6.0. Data pre-processing included removal of features with > 20 % missing values, gap-filling by k-nearest neighbours (k = 5), $\log_2 (x + 1)$ transformation and autoscaling (zero mean, unit variance). Univariate analyses were performed on normalized means using one-way ANOVA (analysis of variance) or Kruskal-Wallis tests when parametric assumptions were

not met. P-values were adjusted via the Benjamini–Hochberg method, with metabolites considered significant at FDR < 0.05. Multivariate analyses were conducted on the scaled dataset. Principal component analysis (PCA) was used to visualize sample clustering and detect discriminating metabolites, with the first two principal components (PCs) explaining ~54 % of total variance. Group separation was evaluated by pairwise t-tests (Holm-adjusted p-values) and confirmed using PERMANOVA (999 permutations). PCA loadings were examined to identify top contributors. Hierarchical clustering (Euclidean distance, Ward's linkage) was applied to visualize abundance patterns. Random Forest classification (500 trees, mtry = √p) ranked variable importance by mean decrease in accuracy and model performance was validated by out-of-bag error rates and cross-validation.

Results and Discussion

Characterisation of cow urine

Basic characterization of raw cow urine revealed that it has alkaline pH of 9.63-9.95, high EC of 23.80 dS/m-24.90 dS/m and contained 2.8 %-3.0 % organic carbon (OC) and was rich in N (0.52 %-0.76 %), with low P and K. Among macronutrients, Ca (0.078 %-0.080 %), Mg (0.029 mg L^{-1}-0.030 mg L^{-1}), sodium, Na (0.48 %-0.50 %) and S (1.6 %-2.2 %) were notable. Micronutrient analysis showed the presence of Fe (10 mg L^{-1}) , Mn (0.5 mg L^{-1}), Zn (0.1 mg L^{-1}), Cu (0.05 mg L^{-1}) and Ni (0.1 mg L^{-1}). Trace amounts of chromium (Cr), Pb and cadmium (Cd) (< 0.05 mg L^{-1}) were also detected.

Qualitative screening shows the presence of flavonoids, terpenoids, proteins, quinones, phenols, tannins and glycosides (Table 1). Further the untargeted metabolomic profiling by LC-MS/MS confirms the specific compounds belongs these classes (Table 2). Untargeted LC–MS/MS analysis of cow urine revealed a diverse metabolite profile comprising phenolic acids, flavonoids, glycosides, amino acids, terpenoids, lipids, alkaloids and other bioactive compounds. Phenolic acids and their derivatives were predominant, including protocatechuic acid (19.11 %), vanillic acid (33.7 %), gallic acid (24.61 %), syringic acid (42.69 %), isoferulic acid (21.53 %) and various conjugated forms such as coumaric acid hexose (71.16 %), coumaric acid sulfate (43.02 %) and ferulic acid-O-hexoside (11.43 %). Flavonoid compounds such as epicatechin (15.81 %), gallocatechin (10.17 %), quercetin fragments (19.44 %), myricetin-O-hexoside (10.02 %), luteolin 7-O-glucuronide (6.83 %) and apigenin 6-C-glucoside (10.65 %) were also detected. Several glycosides including iridoid, phenolic, steroidal, chalcone and triterpenoid types were present, along with diterpenes, sphingolipids (myriocin), bile acid derivatives (glycocholic acid) and phytohormone related metabolites (isopentenyladenine-9-N-glucoside). The high relative intensities of phenolic acids and flavonoids suggest a strong antioxidant potential, while the occurrence of diverse glycosides, terpenoids and alkaloids points to a broad spectrum of biological activities in cow urine. Additionally, compounds of pharmacological and antimicrobial relevance such as tuberonic acid glucoside, isoliquiritin, lusitanicoside, melampodinin and beta-peltatin A methyl ether were identified.

Untargeted metabolomes profiling of botanical extracts

LC–MS/MS TIC (Total Ion Chromatogram) runs in both positive and negative ion modes are presented in Fig. 1–7. But in the *M. indica* water (MIW) extract, TIC compounds were detected exclusively in negative ion mode, with no detectable peaks in positive ion mode. LC-MS/MS profiling revealed that cow urine contained the highest number of 316 compounds in negative ionization mode $[M-H]^-$, with 38 in positive mode [M+H] and 22 compounds in both modes. Among the extracts, LCCU (cow urine extract of *L. camara*) showed the highest number of 274, of positive mode compounds while LCW (water extract of *L. camara*) recorded the highest of 300 compounds in negative mode. CCCU (cow urine extract of *C. citratus* (DC.) Stapf.), CCW (water extract of *C. citratus*, MICU (cow urine extract of *M. indica*) and MIW (water extract of *M. indica*) exhibited variable profiles, with MICU rich in positive mode has 186 compounds but none detected in positive mode for MIW (Supplementary Table 1-6). Overall, cow urine displayed the most diverse metabolite profile in negative mode compared to other treatments. The metabolomic profiling of six extracts viz. CCCU, CCW, LCCU, LCW, MICU and MIW revealed distinct phytochemical compositions and clear group separation.

PCA revealed clear separation among the six sample groups (CCCU, CCW, LCCU, LCW, MICU and MIW) based on their metabolite profiles (Fig. 8). Cow urine extracts formed discrete groups away from water extracts, highlighting the influence of extraction medium on metabolite profiles. The first two principal components (PC1: 29.1 %, PC2: 25 %) together explained 54.1 % of the total variance, while the first five PCs cumulatively accounted for 100 % of the variance. Pair wise statistical comparisons showed significant differences (p = 0.001) for PC1 vs PC2, PC1 vs PC5 and PC2 vs PC3, while other

Table 1. Qualitative detection of phytoconstituents in cow urine and botanicals

Metabolite	Test performed	Cow urine	CC	LC	MI
Alkaloids	+Dragendorff's reagent test	-	+	+	+
Flavonoids	Alkaline test	+	+	-	+
	+ H_2SO_4	-	-	+	-
Sterols	+ $CHCl_3$ + Acetic anhydride + Conc. H_2SO_4	-	+	-	+
Terpenoids	+ $CHCl_3$ + Acetic anhydride + Conc. H_2SO_4	+	+	+	+
Anthraquinone	+ $FeCl_3$ + Conc. HCl+ diethyl ether + Ammonia	-	+	+	+
Anthocyanin	HCl Test	-	-	-	-
Proteins	+2 % Ninhydrin test	+	+	+	+
	+ conc. HNO_3	+	-	+	-
Phenolic compounds	+5 % neutral $FeCl_3$	+	+	+	+
Quinones	Conc. HCl	+	-	-	-
Carbohydrates	Molisch's test	-	+	+	+
	Fehling's test	-	-	+	+
Tannin	Braymer's test	+	+	+	+
Saponins	Shaken with water	-	+	+	+
Glycoside's test	Borntrager's test	+	+	+	+
	Aqueous NaOH test	-	-	+	-
Coumarins	+ 10 % NaOH + $CHCl_3$	-	-	+	-
Volatile oils	Fluorescence test		+	-	-

*(+) and (-) indicates the presence and absence of compounds respectively.

Table 2. Untargeted metabolites identified in cow urine alone by LC-MS/MS

Ionization	Relative intensity %	M/Z	Compound name	Chemical class
[M-H]-	19.11	154.26	protocatechuic acid	Phenolic acid
[M-H]-	33.7	160.16	vanilic acid	Phenolic acid
[M-H]-	79.26	162.01	7-hydroxycoumarin	Coumarin
[M-H]-	71.16	164.01	p-coumaric acid hexose	Phenolic glycoside
[M-H]-	34.33	166.06	phenylalanine	Amino acid
[M-H]-	24.61	168.21	gallic acid	Phenolic acid
[M-H]-	19.11	182.21	benzoic acid, 2,4-dimethoxy-	Phenolic acid derivative
[M-H]-	14.69	184.26	phospholipids derivative	Lipid
[M-H]-	10.81	194.16	isoferulic acid	Phenolic acid
[M-H]-	51.97	197.06	gallic acid methyl ester	Phenolic acid ester
[M-H]-	42.69	198.96	syringic acid	Phenolic acid
[M-H]-	15.81	207.11	epicatechin	Flavan-3-ol
[M-H]-	9.85	209.06	zingerone	Phenolic ketone
[M-H]-	43.02	244.16	coumaric acid sulfate	Phenolic acid sulfate
[M-H]-	12.52	258.26	caffeic acid 4-sulfate	Phenolic acid sulfate
[M-H]-	8.82	268.21	3-hydroxydaidzein	Isoflavone
[M-H]-	19.14	272.31	3-O-methylequol	Isoflavone derivative
[M-H]-	19.44	304.36	quercetin fragment	Flavonol fragment
[M-H]-	39.25	306.16	(epi)catechin	Flavan-3-ol
[M-H]-	10.17	308.26	Gallocatechin	Flavan-3-ol
[M-H]-	10.65	314.31	apigenin 6-C-glucoside	Flavone glycoside
[M-H]-	10.02	318.36	myricetin-O-hexoside	Flavonol glycoside
[M-H]-	12.65	322.21	phenolic derivative	Phenolic compound
[M-H]-	14.45	324.31	phenolic derivative	Phenolic compound
[M-H]-	15.31	326.36	coumaric acid derivative	Phenolic acid derivative
[M-H]-	31.96	330.31	vanillic acid hexoside	Phenolic glycoside
[M-H]-	13.57	340.31	diterpene	Diterpenoid
[M-H]-	10.74	342.31	Caffeoyl glycoside	Phenolic glycoside
[M-H]-	10.81	344.31	caffeoyl hexoside	Phenolic glycoside
[M-H]-	13.97	346.26	diterpene	Diterpenoid
[M-H]-	11.43	356.36	ferulic acid-O-hexoside	Phenolic glycoside
[M-H]-	11.64	358.31	diterpene	Diterpenoid
[M-H]-	5.79	363.31	isopentenyladenine-9-N-glucoside	Cytokinin glycoside
[M-H]-	12.71	372.31	syringin	Phenylpropanoid glycoside
[M-H]-	21.21	374.21	deoxyloganin	Iridoid glycoside
[M-H]-	8.87	385.26	buspirone	Azaspirodecanedione derivative
[M-H]-	13.83	388.31	tuberonic acid glucoside	Fatty acid glycoside
[M-H]-	10.4	390.26	rehmaionoside A	Iridoid glycoside
[M-H]-	10.25	395.26	steroidal glycosides	Steroidal glycoside
[M-H]-	10.31	400.36	myriocin	Sphingolipid
[M-H]-	16.43	418.21	isoliquiritin	Chalcone glycoside
[M-H]-	11.36	428.31	beta-peltatin A methyl ether	Lignan
[M-H]-	11.2	440.31	10-deacetyl-2-debenzoylbaccatin III	Diterpenoid alkaloid
[M-H]-	9.18	442.31	lusitanicoside	Phenylethanoid glycoside
[M-H]-	6.83	462.31	luteolin 7-O-glucuronide	Flavone glycoside
[M-H]-	7.04	465.26	glycocholic Acid	Bile acid glycoside
[M-H]-	10.97	491.36	demethylalangiside	Triterpenoid glycoside
[M-H]-	9.66	492.31	aurantio-obtusin beta-D-glucoside	Anthraquinone glycoside
/[M-H]-	7.33	522.31	melampodinin	Sesquiterpene lactone
[M-H]-	5.27	546.31	ergosine	Ergot alkaloid
[M+H]+	40.28	164.14	p-coumaric acid hexose	Phenolic glycoside
[M+H]+	11.22	166.19	L-Phenylalanine	Amino acid
[M+H]+	5.64	186.14	furo(4',5',6,7)coumarin	Coumarin derivative
[M+H]+	17.26	189.09	oleanolic acid	Triterpenoid
[M+H]+	21.53	194.09	isoferulic acid	Phenolic acid
[M+H]+	17.65	198.14	ethyl galate	Phenolic acid ester
[M+H]+	7.1	205.09	guanidine compound	Guanidine compound
[M+H]+	52.31	212.14	Brimonidine	Imidazoline derivative
[M+H]+	12.71	232.19	3/4-hydroxyphenyl acetic acid sulfate	Phenolic acid sulfate
[M+H]+	6.33	270.19	dalbergin	Neoflavonoid
[M+H]+	11.14	310.14	coumaric acid derivative	Phenolic acid derivative

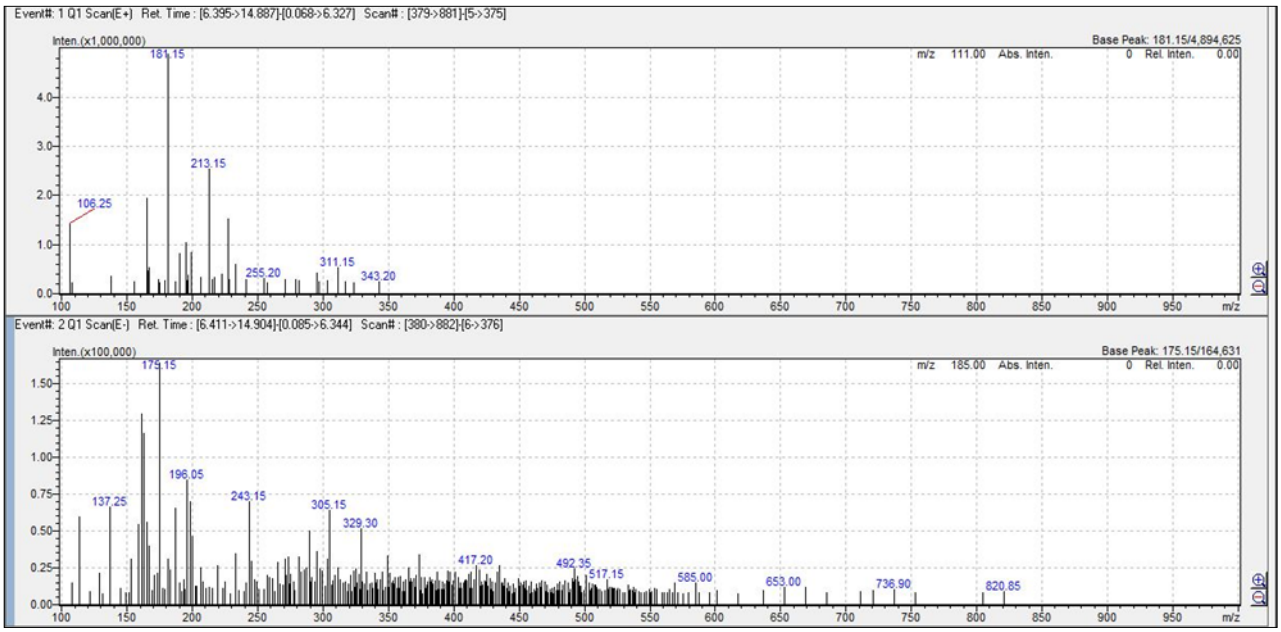


Fig. 1. LC-MS/MS chromatogram showing the m/z of ions (metabolites) detected in cow urine.

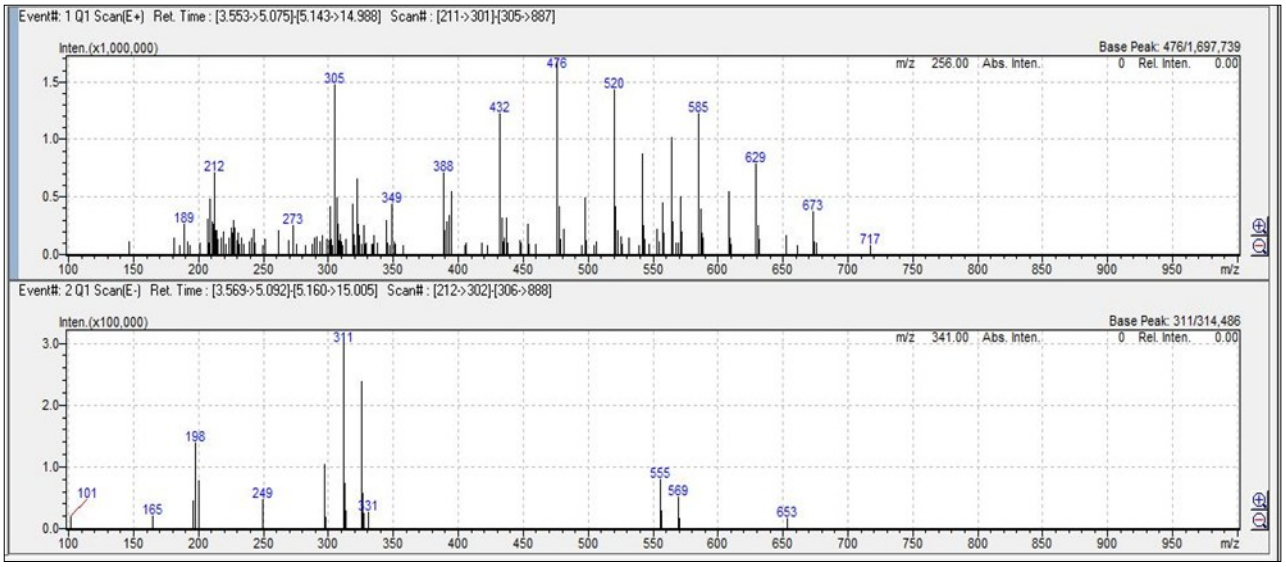


Fig. 2. Chromatogram showing the non-targeted metabolites in cow urine extract of *C. citratus* determined by LC-MS/MS in both positive and negative mode.

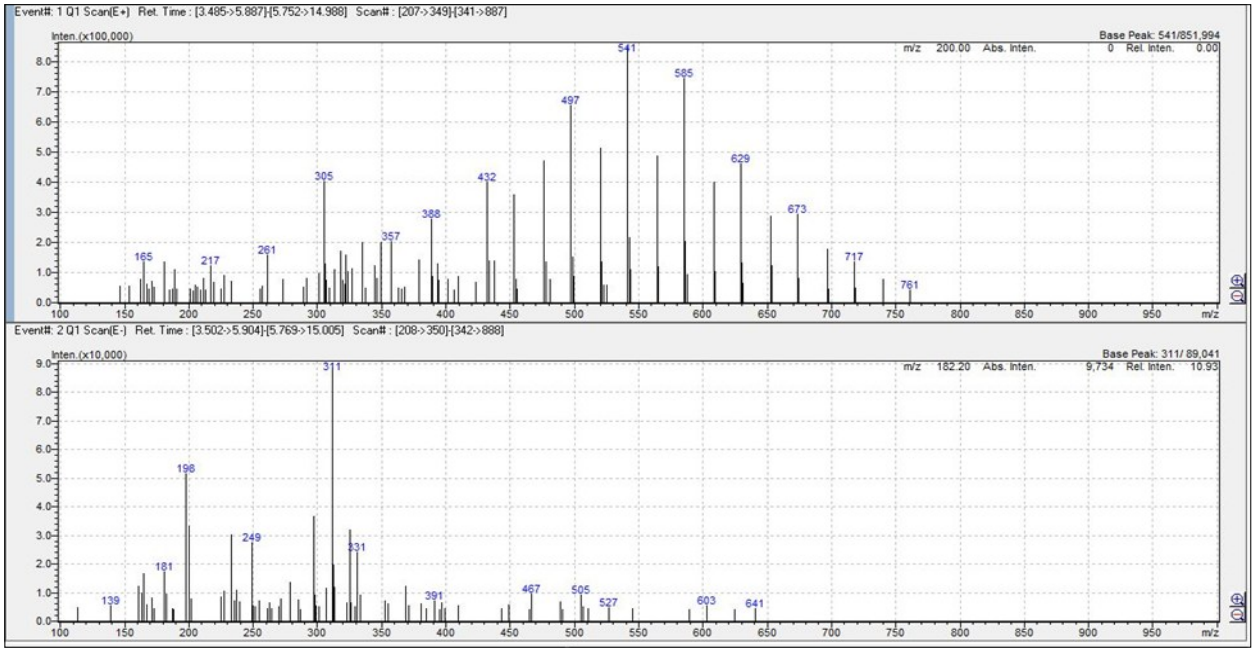


Fig. 3. Chromatogram showing the non-targeted metabolites in water extract of *C. citratus* determined by LC-MS/MS in both positive and negative mode.

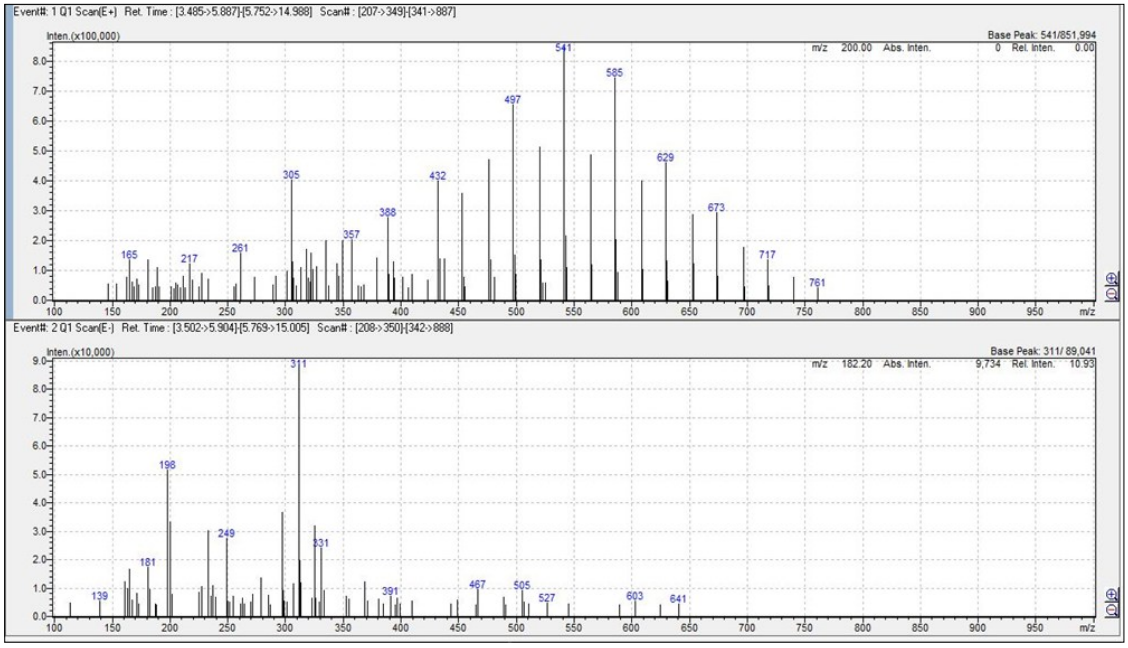


Fig. 4. Chromatogram showing the non-targeted metabolites in cow urine extract of *L. camara* determined by LC-MS/MS in both positive and negative mode.

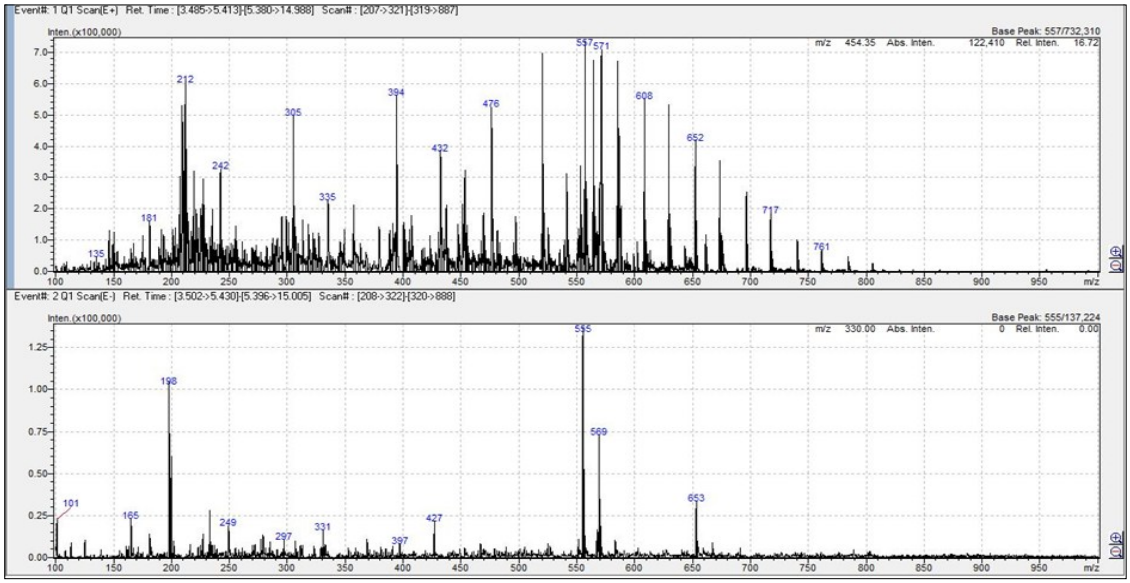


Fig. 5. Chromatogram showing the non-targeted metabolites in water extract of *L. camara* determined by LC-MS/MS in both positive and negative mode.

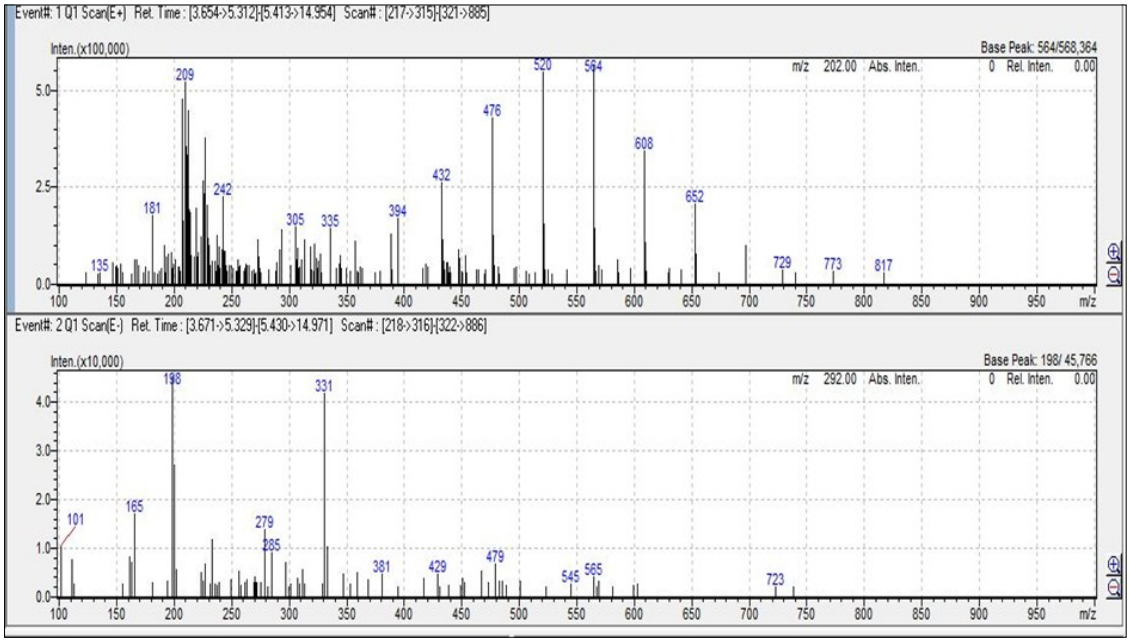


Fig. 6. Chromatogram showing the non-targeted metabolites in cow urine extract of *M. indica* determined by LC-MS/MS in both positive and negative mode.

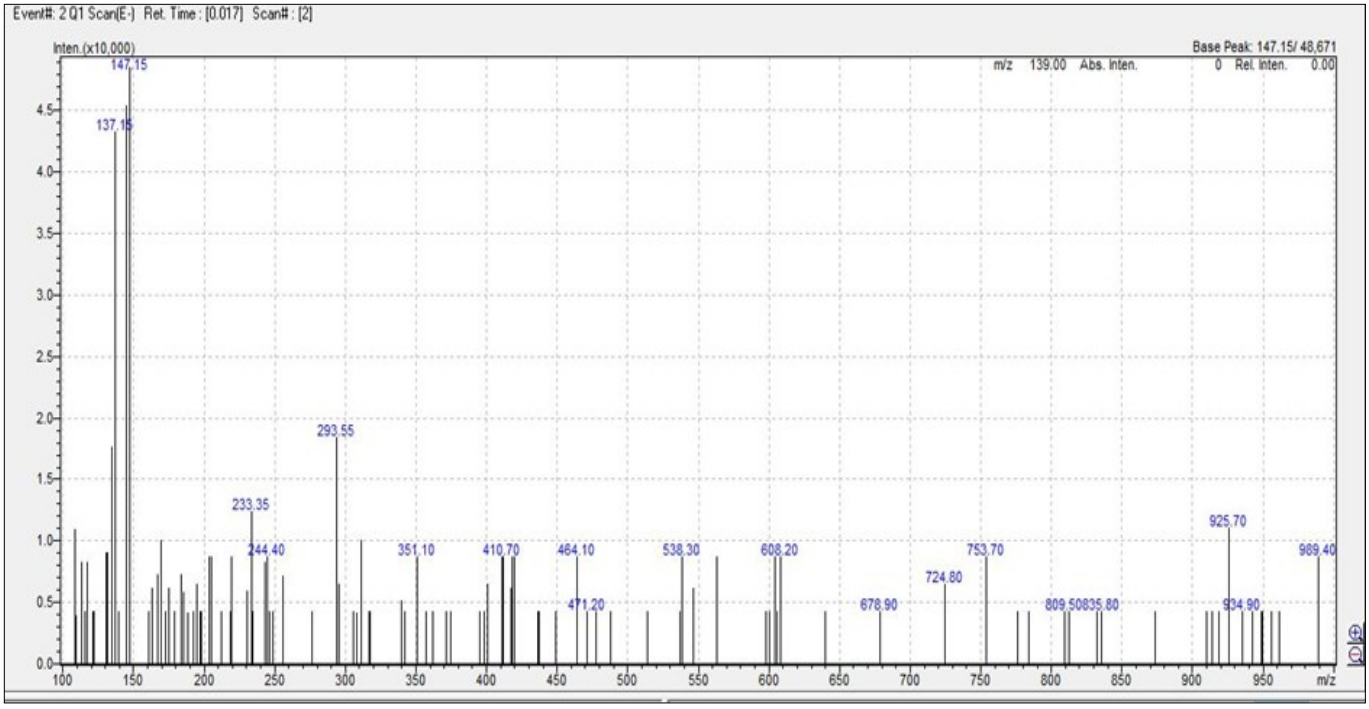
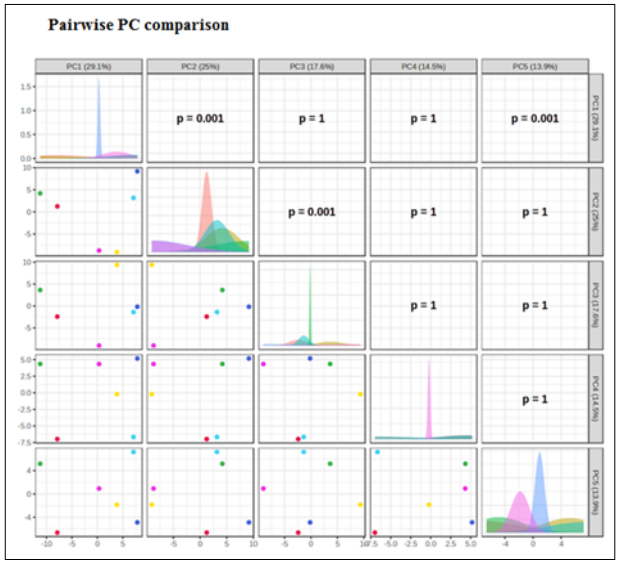
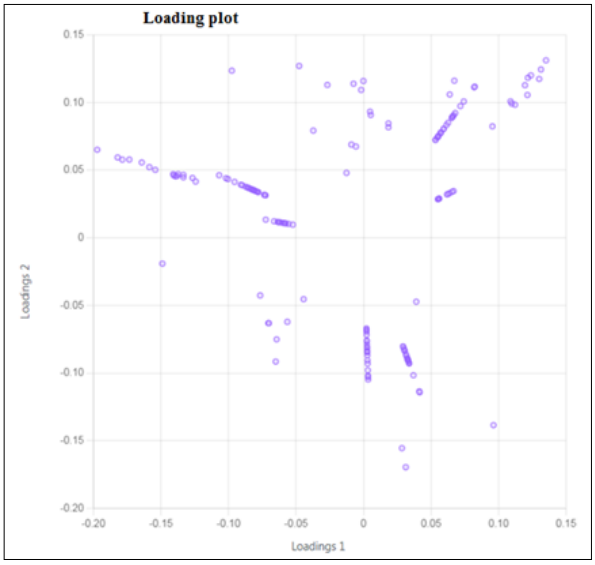
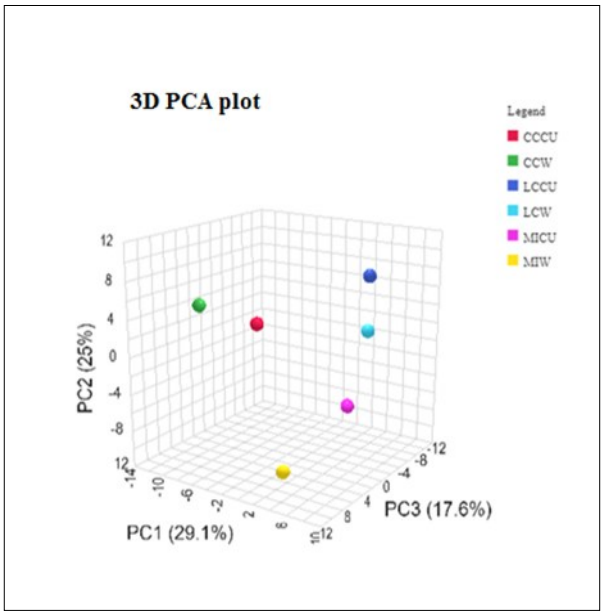


Fig. 7. Chromatogram showing the non-targeted metabolites in water extract of *M. indica* determined by LC-MS/MS in negative mode.







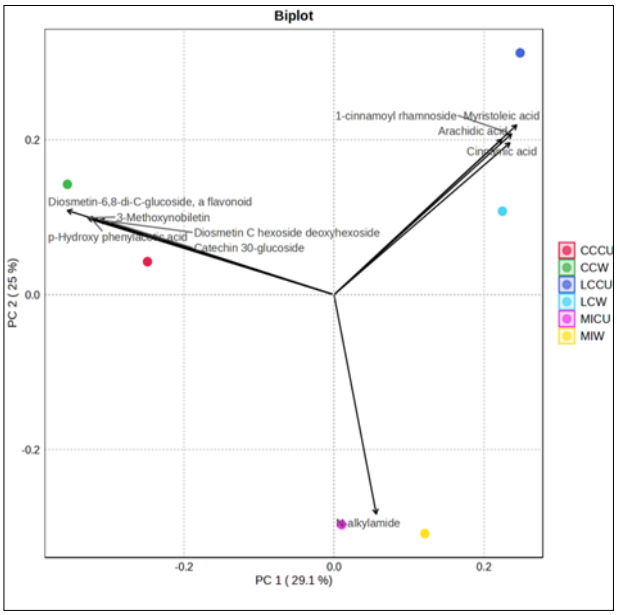


Fig. 8. PCA of metabolomes detected in botanical extracts by LC-MS/MS.

combinations were not statistically different (p = 1). The loadings plot indicated distinct clusters of features with both positive and negative contributions to PC1 and PC2. The 3D PCA plot demonstrated distinct spatial clustering of the six groups, with minimal overlap. The biplot further revealed that LCCU and LCW were associated with higher levels of 1-cinnamoyl rhamnoside, myristoleic acid, arachidic acid and cinnamic acid; MICU was characterized by alkylamide and CCCU and CCW were linked to flavonoids such as diosmetin-6,8-di-C-glucoside, 3-methoxynobiletin, p-hydroxyphenylacetic acid, diosmetin-C-hexoside-deoxyhexoside and catechin 3-O-glucoside. These results indicate that the identified metabolites contribute substantially to the observed group differentiation. The discussion integrates both the experimental data and literature-supported observations to contextualize the findings. Referencing known antimicrobial properties of cow urine and lemongrass activity helps explain the observed bioactivity and supports the potential mechanisms behind the weed suppression and nutrient enrichment reported in this study.

Since the consolidated heat map was extensive, hierarchical clustering heat map analyses were performed separately for phenolics, flavonoids, terpenoids and other miscellaneous bioactive metabolites (Fig. 9a-d). These analyses revealed clear, treatment-specific chemical signatures across the six botanical extracts. Cow urine-based extracts consistently exhibited greater metabolite diversity and abundance than water-based extracts, highlighting their superior extraction efficiency for both polar and non-polar phytochemicals. Among them, CCCU formed a distinct cluster, enriched in a broad phenolic spectrum (syringic acid, sinapic acid derivatives, chlorogenic acid, ferulic acid, p-coumaric acid, gallic acid) alongside high levels of flavonoids such as apigenin derivatives, luteolin glycosides, kaempferol derivatives, rutin, catechin and epicatechin, as well as notable lipophilic terpenoids. Its water-based counterpart, CCW, shared some of these compounds but at reduced abundance. LCCU stood out as the most chemically diverse extract, being strongly enriched in lantadenes (A, C, D, E), lantic acid, pomolic acid, lantanoside, ursane and oleanane type triterpenes, diosmetin derivatives, kaempferide, flavonoid pentosides, citral, camarin, pheophorbide derivatives, cinnamic acid, multiple methyl ester fatty acids and rare alkaloids such as N-hydroxylysine and isomangiferin. LCW contained some of these metabolites but in lower concentrations. MICU was particularly rich in hydroxybenzoic acid derivatives, gallic acid conjugates, caffeoyl derivatives, kaempferol-related flavonoids, catechin gallate, epicatechin gallate, naringenin and various phenolic acids, flavonoids and benzophenones, whereas MIW showed the lowest metabolite diversity, containing only selected alkaloids, amino acids (L-phenylalanine), indole- and oleanolic acid derivatives. Overall, the clustering patterns clearly demonstrate that cow urine extraction enhances the solubilization and recovery of a wider spectrum of phytochemicals-including

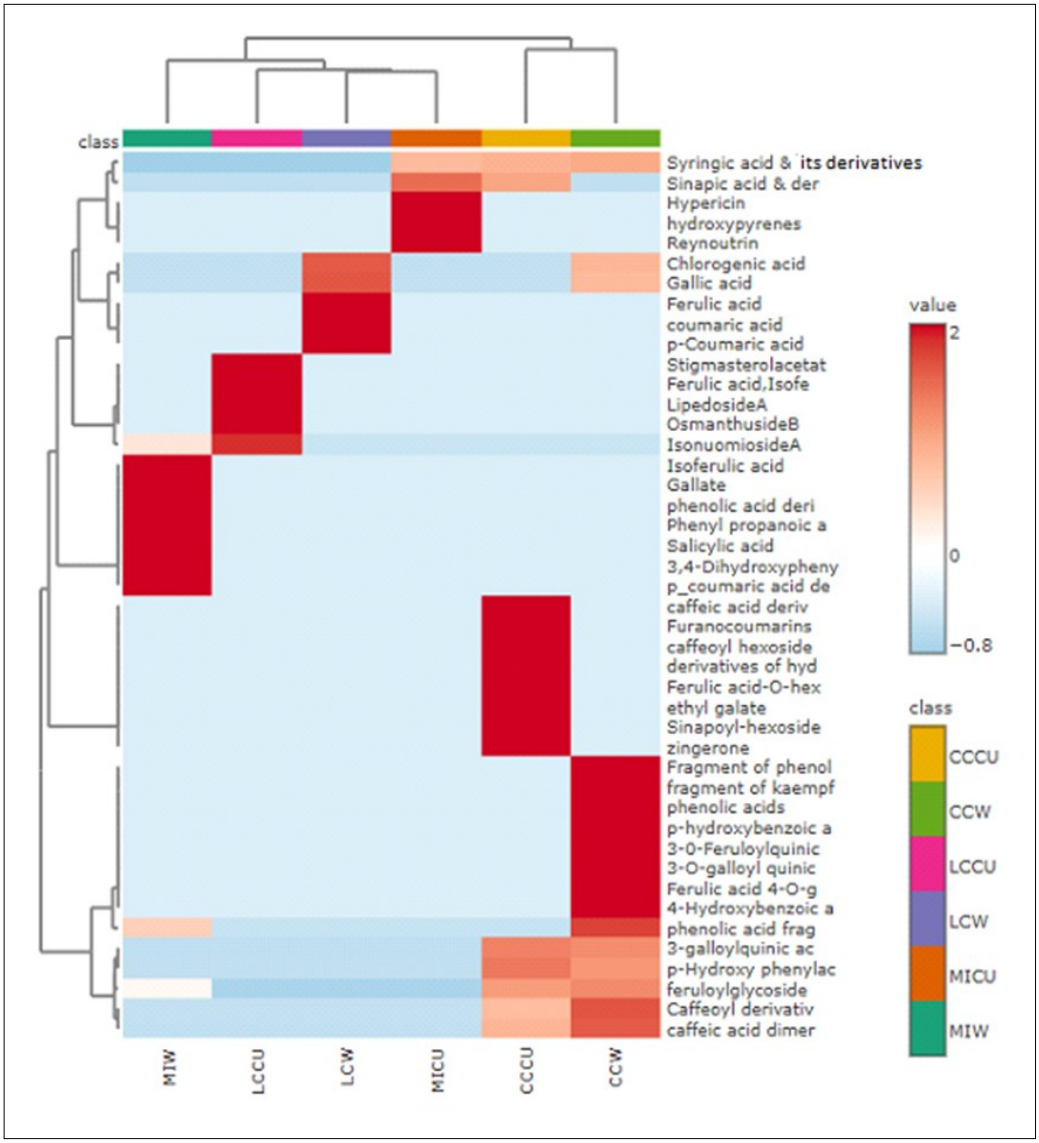


Fig. 9a. Heat map illustrating the distribution of phenolic compounds and related metabolites in cow urine and botanical water extracts.

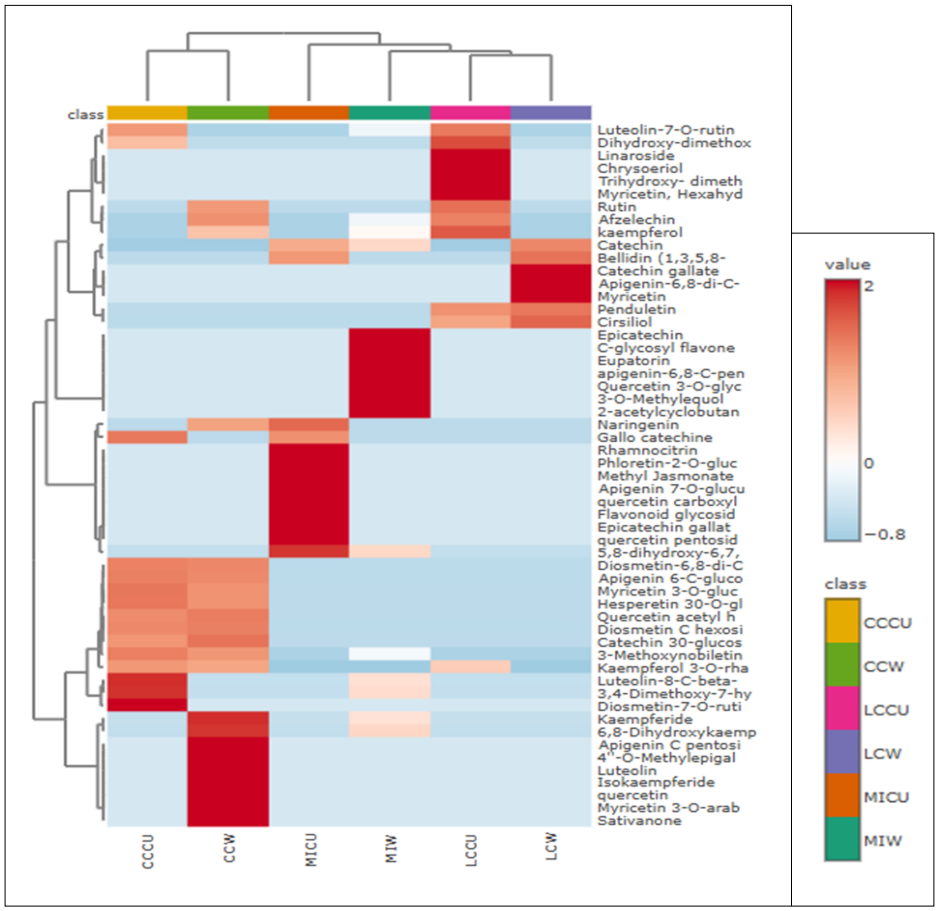


Fig. 9b. Heat map depicting the distribution of flavonoids and their related metabolites in cow urine and botanical water extracts.

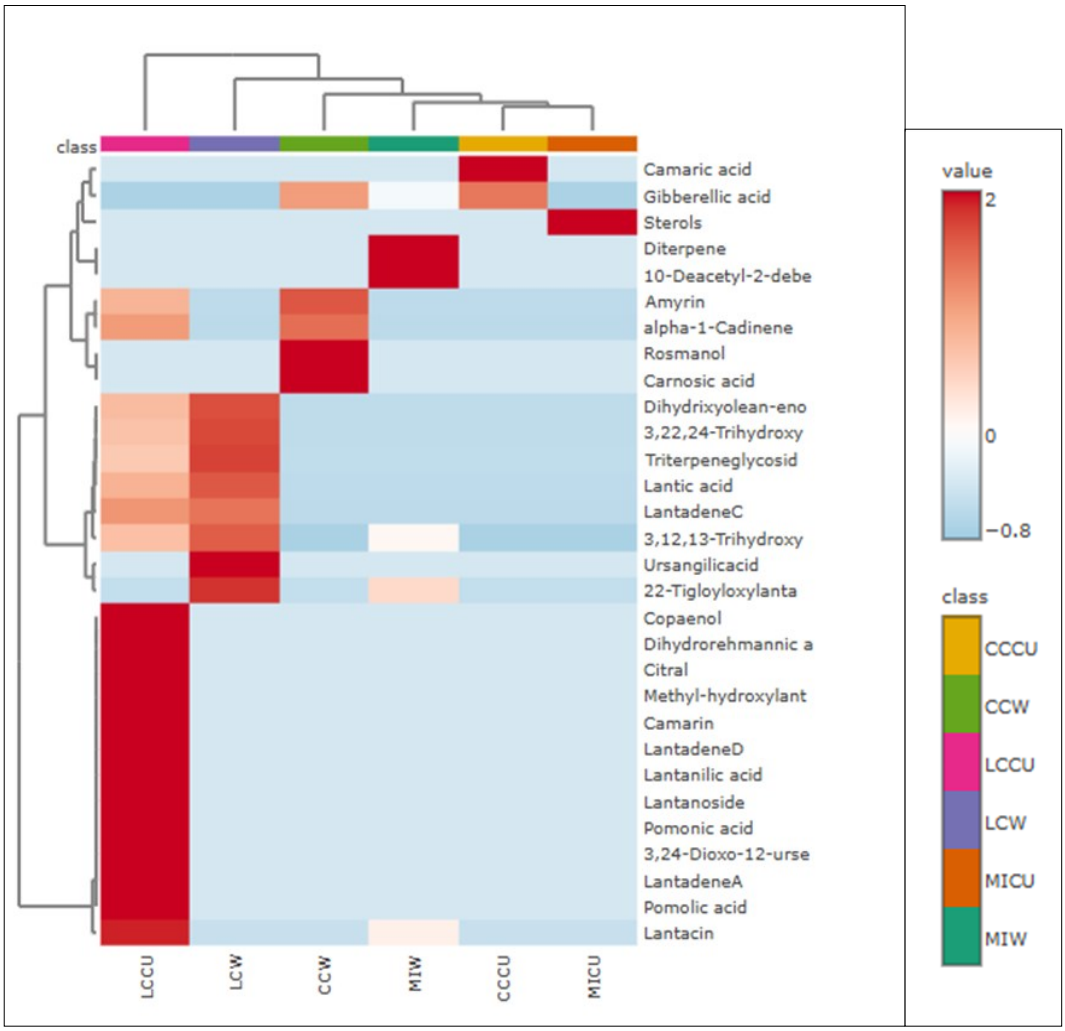


Fig. 9c. Heat map illustrating the distribution of terpenoids and their related metabolites in cow urine and botanical water extracts.

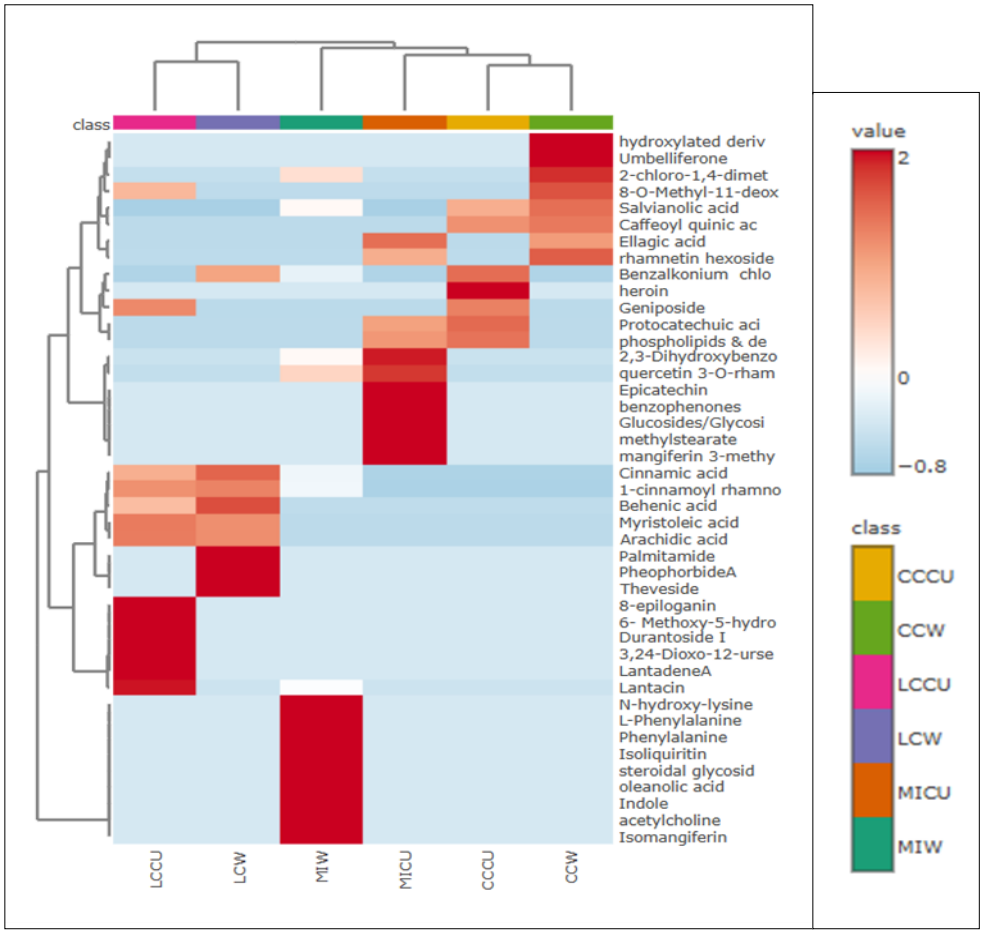


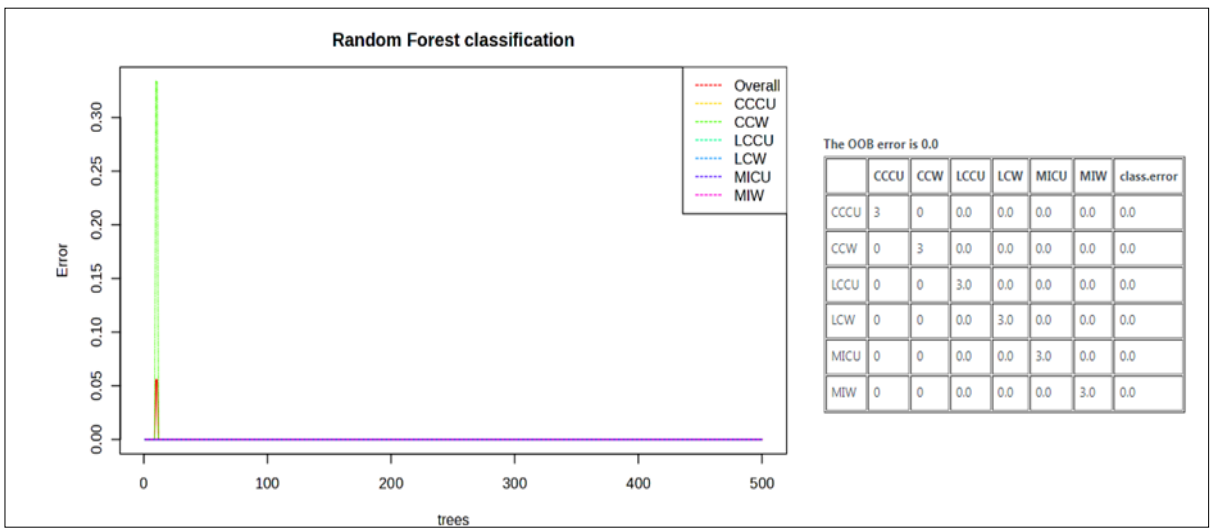
Fig. 9d. Heat map depicting the distribution of miscellaneous metabolites, including fatty acids and amino acids, in cow urine and botanical water extracts.

phenolics, flavonoids, terpenoids, alkaloids and fatty acid derivatives -which may underpin the superior weed suppression, nutrient enrichment and broader bioactivity observed in these treatments.

Random Forest classification (Fig. 10) achieved a perfect classification accuracy (OOB error = 0.0 %), with zero misclassification across treatments. This indicates that the phytochemical composition of each extract type was distinct enough to allow complete separation, with certain marker metabolites contributing strongly to class discrimination. These results demonstrate that cow urine as an extraction medium significantly alters the metabolite composition of plant extracts, enriching bioactive compounds with potential allelopathic activity. Such phytochemicals including flavonoids, phenolic acids and terpenoids are known to interfere with weed germination and growth by disrupting cell division, membrane integrity and oxidative balance in target plants.

Mineral composition of botanical extracts

The mineral composition of the botanical extracts varied substantially depending on plant species and extraction medium (Table 3). OC content was highest (0.618 %) in CCCU and lowest (0.045 %) in LCW. A higher OC content, as seen in cow urine extracts, can improve soil microbial activity and enhance nutrient availability for plants. Nitrogen concentration peaked in MICU (0.364 %), followed by CCCU (0.280 %), while LCW recorded the lowest value (0.1601 %). Elevated N is



	CCCU	CCW	LCCU	LCW	MICU	MIW	class.error
CCCU	3	0	0.0	0.0	0.0	0.0	0.0
CCW	0	3	0.0	0.0	0.0	0.0	0.0
LCCU	0	0	3.0	0.0	0.0	0.0	0.0
LCW	0	0	0.0	3.0	0.0	0.0	0.0
MICU	0	0	0.0	0.0	3.0	0.0	0.0
MIW	0	0	0.0	0.0	0.0	3.0	0.0

Fig. 10. Random forest analysis of non-targeted metabolomes detected by LC-MS/MS.

Table 3. Mineral composition of botanical extracts

Mineral composition	CCCU	CCW	LCCU	LCW	MICU	MIW
OC (%)	0.618 ± 0.001	0.075 ± 0.003	0.075 ± 0.001	0.045 ± 0.00	0.362 ± 0.002	0.06 ± 0.00
N (%)	0.280 ± 0.001	0.224 ± 0.007	0.224 ± 0.007	0.160 ± 0.001	0.364 ± 0.007	0.20 ± 0.005
P (%)	0.0044 ± 0.00	0.004 ± 0.00	0.0001 ± 0.00	0.0028 ± 1.239	0.005 ± 0.00	0.0035 ± 0.00
K (mg L^{-1})	17.4 ± 0.471	86.6 ± 0.546	52.0 ± 1.172	55 .0 ± 0.130	43.8 ± 0.316	70 ± 1.388
Ca (mg L^{-1})	374.85 ± 9.799	6.2 ± 0.235	633.45 ± 9.136	4 ± 0.246	257.2 ± 1.159	5.5 ± 0.079
Mg (mg L^{-1})	275.65 ± 3.975	10.6 ± 0.115	421.4 ± 14.814	7 ± 0.00	375.6 ± 4.740	9 ± 0.081
S (%)	0.053 ± 0.002	0.048 ± 0.00	0.077 ± 0.001	0.030 ± 0.379	0.068 ± 0.001	0.04 ± 0.001
Na (mg L^{-1})	1669.7 ± 69.23	109.2 ± 1.476	4586.15 ± 12.402	60 ± 0.052	4986.4 ± 121.36	90 ± 1.947
Fe (mg L^{-1})	1.5 ± 0.061	3 ± 0.041	6.85 ± 0.074	2 ± 0.002	4.7 ± 0.169	2.5 ± 0.086
Zn (mg L^{-1})	0.1 ± 0.001	0.1 ± 0.001	0.1 ± 0.004	0.05 ± 0.00	0.25 ± 0.009	0.08 ± 0.00
Mn (mg L^{-1})	0.2 ± 0.002	ND	0.1 ± 0.002	ND	0.25 ± 0.005	0.1 ± 0.001
Cu (mg L^{-1})	0.1 ± 0.004	ND	0.15 ± 0.003	ND	0.15 ± 0.001	0.05 ± 0.001
Ni (mg L^{-1})	0.1 ± 0.003	ND	0.1 ± 0.004	ND	0.2 ± 0.001	0.05 ± 0.001
Cr (mg L^{-1})	0.05 ± 0.001	0.1 ± 0.002	0.05 ± 0.001	0.05 ± 0.05	0.05 ± 0.001	0.08 ± 0.002
Pb (mg L^{-1})	0.1 ± 0.002	ND	0.1 ± 0.004	ND	0.2 ± 0.003	0.05 ± 0.001
Cd (mg L^{-1})	0.05 ± 0.002	ND	ND	ND	0.05 ± 0.001	0.01 ± 0.001

*± Standard deviation values; ND: Not detected.

particularly important for chlorophyll formation and vegetative growth, suggesting that MICU may have a stronger role in promoting biomass accumulation. P content was generally low across all treatments, with the highest value in MICU (0.005 %) and the lowest in LCCU (0.0001 %). Even at low levels, P is vital for root development and energy transfer, indicating that MICU extracts could still contribute to early root establishment. K levels ranged from 17.4 ± 0.471 mg L^{-1} in CCCU to 86.6 ± 0.546 mg L^{-1} in CCW. K supports stomatal regulation, stress tolerance and its higher concentration in CCW may benefit plants under water stress conditions. Ca and Mg were particularly abundant in LCCU (633.45 ± 9.136 mg L^{-1} and 421.4 ± 14.814 mg L^{-1} respectively). These nutrients play essential roles in cell wall stabilization, membrane integrity and photosynthesis. S content was highest in LCCU (0.077 ± 0.001 %), with MICU also recording elevated levels (0.068 ± 0.001 %). Since S is linked with amino acid synthesis, these extracts may enhance protein formation in plants. Na levels were markedly higher in cow urine extracts, especially MICU (4986.4 ± 121.36 mg L^{-1}) and LCCU (4586.15 ± 12.402 mg L^{-1}), compared to water extracts. While excessive Na can be detrimental, moderate enrichment may influence osmotic regulation. Among micronutrients, Fe was highest in LCCU (6.85 ± 0.074 mg L^{-1}), supporting chlorophyll biosynthesis and preventing leaf chlorosis. Zn and Mn reached peak values in MICU (0.25 mg/L), both of which are critical for enzymatic activation and metabolic regulation. Cu, Ni, Pb and Cd were present in low concentrations, while Mn, Cu, Ni, Pb, Cd were undetectable in the water extracts of MI, LC and CC, thereby reducing potential toxicity risks. Chromium was detected at similarly low levels (0.05 mg L^{-1} - 0.10 mg L^{-1}) in all treatments.

Cow urine extracts, particularly exhibited higher mineral concentrations than their corresponding water extracts, suggesting enhanced solubilization and nutrient enrichment. The abundance of essential macronutrients and micronutrients in these extracts indicates a strong potential to support plant growth, not only by providing readily available nutrition but also by improving physiological functions such as photosynthesis, root establishment, stress tolerance and protein synthesis. N supports chlorophyll synthesis and vigorous vegetative growth, P promotes root development and energy transfer, while K regulates stomatal function and enhances drought tolerance. Ca strengthens cell walls and root tips; Mg is central to chlorophyll and photosynthetic activity; besides, S contributes to amino acid and enzyme formation. Among micronutrients, Fe prevents chlorosis and maintains chlorophyll stability, Zn supports hormone regulation as well as enzyme activation and Mn facilitate photosynthetic electron transport.

Effect of botanical extracts on weeds growth inhibition

WSE varied notably among botanical extracts and increased progressively with applied concentration (Fig. 11). At 25 days after transplanting (DAT), CCCU recorded the highest WSE (31.89 %). Although these values are lower than those typically achieved with

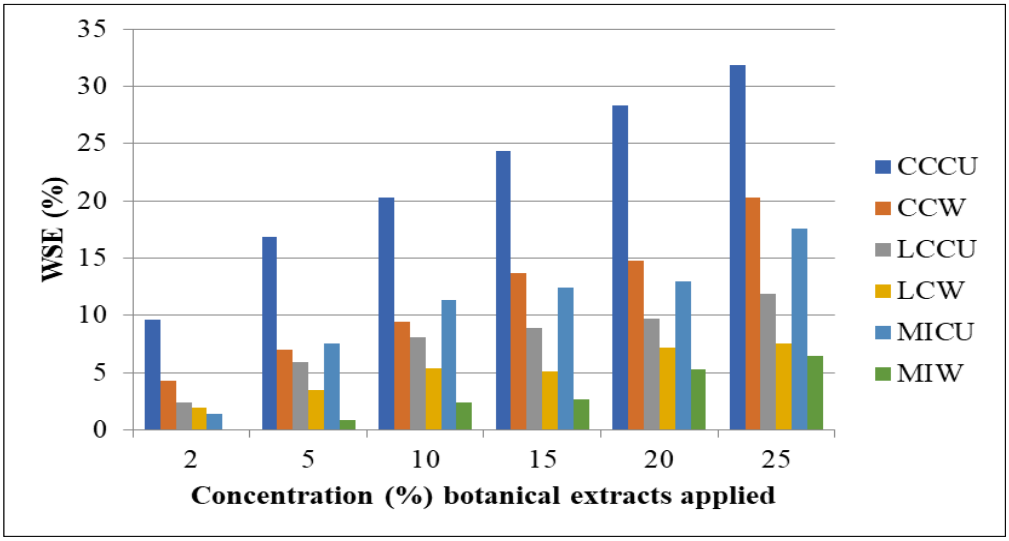


Fig. 11. Effect of cow urine and water based botanical extracts on total weed control efficiency under *in vitro* lab study.

synthetic herbicides, they may still hold agronomic relevance under specific production systems. For instance, in low-input or organic farming, where synthetic herbicide use is restricted, even partial suppression can reduce early-season weed competition and improve crop establishment. Similarly, under conditions of moderate weed pressure or when integrated with cultural practices such as mulching, hand weeding or crop rotation; botanical extracts could contribute to an overall weed management strategy. Thus, while the efficacy may not be sufficient as a standalone approach in high-pressure environments, these extracts have potential utility as complementary tools within sustainable and integrated weed management programs, followed by CCW (20.27 %) and MICU (17.57 %). LCCU showed moderate efficacy (11.89 %), whereas LCW (7.57 %) remained comparatively less effective. Across all intervals, CCCU consistently outperformed other treatments, achieving 9.59 % WSE as early as 2 DAT and steadily increasing thereafter. In contrast, MIW displayed the lowest WSE throughout the observation period, peaking to 6.49 % at 25 % applied concentration.

The WSE of various botanical extracts was also compared with herbicide glyphosate (standard check) and water control and the results showed marked differences among the treatments (Fig. 12). Glyphosate exhibited the 100 % WSE, confirming its strong herbicidal effect, while the untreated control showed no suppression. Among the botanical extracts, CCCU recorded the greatest mean WSE (22 %), followed by CCW (12 %), MICU (11 %), LCCU (8 %), LCW (5 %) and MIW (3 %). Although all cow urine extracts outperformed their corresponding water extracts, their efficiencies remained considerably lower than glyphosate, reflecting only partial weed suppression.

This investigation holds significant relevance for advancing sustainable crop production systems, particularly in the context of organic and natural farming, where reliance on synthetic inputs is minimized. Normally the metabolic profiling and targeted analysis of growth regulating substances in botanicals is essential to assess their role on crop production under organic and natural farming. Major threat to farmers is weed management and utilizing the allelopathic effect of botanicals for this purpose is pinned by many researchers. However, their efficiency on weed control is low compared to synthetic herbicides. Despite that those botanicals may also promotes plant growth which depends on the applied concentration and metabolomes composition. Hence in the present investigation three botanicals that have shown significant control on weeds were organically extracted and subjected non targeted analysis to identify the compounds responsible for bio herbicidal property or as biostimulant (12). Further the extracts were applied over the weeds under *in vitro* lab study to assess their inhibition effect.

Botanical extracts dependent variation on non-targeted metabolomics profile

The metabolomic and multivariate analyses showed a clear, biologically meaningful partitioning of the six treatment classes (CCCU, CCW, LCCU, LCW, MICU, MIW) and identified a small set of metabolites likely responsible for both the weed-inhibitory and growth-promoting effects observed. PCA (PC1-PC3 together explain the majority of variance in the data) and the biplot show that phenylpropanoid-type compounds (cinnamic/cinnamic-derivatives), flavonoid glycosides (diosmetin derivatives, catechin-3-O-glucoside), certain fatty-acids (e.g. myristoleic/arachidic signatures) and N-alkylamides load strongly on different PCs and point toward particular extract classes; this indicates that those metabolites drive the sample separation and therefore are the best candidates for functional bioactivity. The heatmap and clustering reveal class-specific fingerprints: Lantana treatments (LCCU and LCW) and Lemongrass (CCCU/CCW) formed distinctive metabolite clusters, while Mango extracts (MICU/MIW) separated in a different block. A supervised random-forest model trained on the same metabolite matrix achieved perfect class separation (OOB error ≈ 0 in the current dataset), supporting that the chemical profiles are robust and diagnostic of each extract type (i.e. the classes are chemically distinct and consistently sampled).

Cow urine's antimicrobial, antioxidant and immune modulatory properties have been supported in recent studies (3, 13, 14). In particular, metabolomic and peptidomic analyses have identified plant-derived metabolites and antimicrobial peptides in cow urine-explaining its bioactivity (13). Additionally, *in vivo* studies in urine models demonstrated cow urine reduced bacterial load

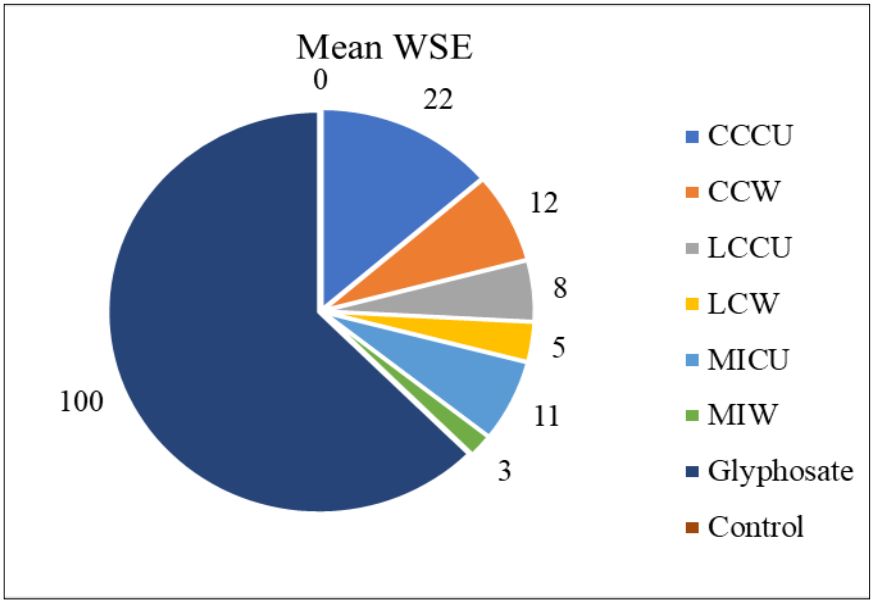


Fig. 12. Effect of cow urine and water-based botanical extracts against glyphosate (standard) and water (control) for their effect on mean weed control efficiency in an *in vitro* laboratory study.

against *Escherichia coli* and *Staphylococcus aureus*, attributed to phenolic and volatile compounds (14). Furthermore, cow urine extracts (CCCU, LCCU, MICU) appear to markedly influence metabolite composition. These metabolomic patterns carry functional implications. The antimicrobial and bioactive potential of lemongrass metabolites (e.g. citral, flavonoids) is well supported (15), while cow urine's augmentation of plant-derived bioactive may enhance therapeutic efficacy, as seen in prior studies (3, 14) on cow urine's synergistic biological effects. Cow urine-based extracts (CCCU, LCCU, MICU) displayed unique clustering and metabolite associations. Existing literature supports cow urine's antimicrobial properties and its role as a bioactive medium rich in diverse compounds (16). These findings rationalize the current observation on clustering of CCCU and CCW (along flavonoid vectors) and the distinct positioning of MICU (with alkylamide) and LCCU / LCW (with cinnamic-acid-related metabolites).

C. citratus , represented by the CCW group, is rich in phenolic compounds, flavonoids and its major bioactive constituent- citral, known for potent antimicrobial, antifungal, anti-inflammatory and antioxidant properties (14). The association of CCW with flavonoid-rich vectors such as diosmetin derivatives and phenylacetic acid in the biplot aligns with this characterization and underscores its expected phytochemical profile. The differentiation of MICU marked by alkylamide presence suggests unique interactions between cow urine and *M. indica* leaf compounds, possibly enhancing or preserving certain lipid-soluble constituents. LCCU and LCW associated with elevated levels of 1-cinnamoyl rhamnoside, myristoleic and arachidic acids and cinnamic acid indicate that *L. camara* extracts may enrich phenolic-acid and fatty-acid profiles differently depending on extraction medium.

Botanical extracts dependent variation in nutrients composition

The mineral composition of botanical extracts was strongly influenced by both plant species and extraction medium. Cow urine extracts consistently contain higher concentrations of macro- and micronutrients than water extracts, reflecting the role of urine as a natural solvent and nutrient source (16). CCCU exhibited the highest OC content, while MICU recorded the greatest N and Na levels, aligning with findings that cow urine can enhance N solubilization and mineral mobilization (17). LCCU was notably rich in Ca and Mg, which are essential for cell wall structure and enzymatic activity in plants (17).

P content was low across all treatments, although MICU and CCW recorded slightly higher values than others, suggesting species-specific release patterns. Elevated S, Zn and Mn levels in MICU indicate potential benefits for chlorophyll synthesis and stress tolerance (18). Micronutrients such as Fe, Cu, Ni were more abundant in cow urine extracts, whereas several elements were absent in water extracts, underscoring the extraction efficiency of cow urine (19).

Overall, the data confirm that cow urine extraction enhances mineral solubilization and enrichment, potentially improving the bioavailability of nutrients when applied as a biostimulant in crop production. These results support earlier reports that cow urine-based formulations improve plant growth and resilience through nutrient supply and bioactive compound delivery (19).

Effect of botanical extracts on WSE

The WSE results reveal a clear dose-dependent response across the tested botanical extracts. CCCU consistently exhibited the highest suppression at all concentrations, increasing from 9.59 % at 2 % concentration to 31.89 % at 25 %. This strong response attributes to the high concentration of potent allelochemicals such as phenolic acids, flavonoids and alkaloids in CCCU as revealed by metabolomic analysis, which exert stronger inhibitory effects on weed germination and growth as application rates increase (20). The steep rise in WSE with concentration indicates both high phytotoxic potential and the absence of any significant inhibitory threshold, meaning efficacy continues to improve without saturation within the tested range. CCW and MICU also showed positive dose–response patterns, with CCW reaching 20.27 % WCE and MICU 17.57 % at 25 % concentration. This suggests that both contain moderately active compounds, possibly terpenoids, saponins and glycosides, which may require higher doses to exert their full phytotoxic effects. LCCU demonstrated intermediate efficacy of 11.89 % at 25 %, indicating the presence of active metabolites but at lower concentrations or with reduced potency compared to CCCU. In contrast, LCW and MIW consistently displayed low suppression of 7.57 % and 6.49 % respectively even at the highest dose, implying either low phytochemical content, poor stability of active molecules or rapid degradation in the test environment. The increasing WSE with concentration across most treatments aligns with typical allelopathic dose-response relationships, where higher concentrations enhance the bioavailability of inhibitory molecules and strengthen their cumulative effect on physiological processes such as seed germination, root elongation, nutrient uptake and photosynthesis (20). Phenolic acids like p-coumaric and ferulic acids can disrupt cell membranes and enzymatic activity, while flavonoids and terpenes can impair chloroplast function and oxidative balance, with effects intensifying at higher doses.

The mean WSE showed a clear disparity between glyphosate (100 %) and botanical extracts, with CCCU achieving the highest suppression (22 %) and MIW the lowest (3 %). Cow urine-based extracts consistently outperformed water extracts, reflecting their greater efficiency in solubilizing bioactive compounds responsible for phytotoxic effects. Nevertheless, even the most effective cow urine extracts achieved less than one-fourth suppression relative to glyphosate. This modest efficacy may be partly explained by the simultaneous release of nutrients during extraction, which could stimulate weed growth and offset the herbicidal effects. Similar findings have been highlighted in allelopathy reviews and field trials, where botanical extracts generally provide only moderate suppression compared with synthetic herbicides (21). Studies using aqueous allelopathic extracts further emphasize their limited effectiveness and the importance of integrating them with cultural practices for sustainable weed management (22).

Overall, the superior performance of CCCU and its distinct dose-response pattern highlights its potential as a natural bioherbicide for sustainable farming systems. Its activity at lower concentrations, with additional improvements at higher doses, indicates adaptability for use under varying weed pressures and crop sensitivities. These results suggest that botanical extracts are more appropriate as complementary tools in organic and low-input systems, where the aim is to suppress rather than completely eradicate weeds. Their effectiveness can be further enhanced through repeated applications and integration with cultural

practices, strengthening their role in sustainable weed management.

Conclusion

This study demonstrated that cow urine-based botanical extracts, particularly CCCU, offer significant weed-suppressive potential alongside enhanced nutrient enrichment. The higher phenolic and flavonoid content in CCCU correlated with superior weed suppression, while its elevated macro- and micronutrient concentrations suggest additional plant growth-promoting benefits. Cow urine extraction proved more effective than water in solubilizing bioactive compounds and minerals, with MICU and LCCU also showing high nutrient levels. Although the WSE observed was relatively low compared to synthetic herbicides, these botanical extracts hold promise when integrated with other management practices such as mulching, crop rotation and manual weeding in organic and low-input systems. Their role is therefore more complementary than standalone in weed management. The superior performance of cow urine extraction over water highlights its ability to enhance the solubility of bioactive compounds and minerals, thereby releasing greater amounts of phytochemicals and nutrients from the plant material. While this study was limited to a single application, repeated applications at 20 days intervals are suggested to further improve weed suppression. Future research should focus on exploring synergistic botanical combinations, optimizing application methods as well as dosages and evaluating potential biostimulant effects on crop yield and quality. Long-term studies are also required to assess environmental safety, soil health impacts and integration into broader weed management frameworks to support large-scale adoption in sustainable farming systems.

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Authors' contributions

MML and PJ wrote the main manuscript, RK and MS edited the draft manuscript, EP, VV processed the data and MRL edited the figures. All authors reviewed the manuscript and approved the final version.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve language. After using this tool/service, the author(s) reviewed and edited the content as needed and take (s) full responsibility for the content of the publication.

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