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Eco Friendly Weed Control Using Cardiospermum halicacabum

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Abstract

The growing reliance on chemical herbicides has sparked concerns about their environmental and health impacts, driving the search for eco-friendly alternatives. Excessive use of chemical fertilizers results in a surplus of nutrients such as nitrogen and phosphorus, which pollutes the soil and contaminates water bodies. This leads to eutrophication, oxygen depletion, and harm to aquatic organisms. Additionally, nitrates from fertilizers can leach into groundwater, creating serious health risks for humans.In response to these challenges, this study explores the potential of Cardiospermum halicacabum (Mudakkathan), a medicinal plant, as a natural herbicide. The research aims to isolate, evaluate, and harness the bioactive components of crude extracts from C. halicacabum leaves, assessing their herbicidal properties against common agricultural weeds. Using ethanol-based and aqueous extraction techniques, crude extracts were obtained and subjected to bioassay-guided fractionation. Advanced techniques like Gas Chromatography-Mass Spectrometry (GC-MS) identified key bioactive compounds, including saponins, alkaloids, and flavonoids. These compounds exhibited significant phytotoxic effects, inhibiting seed germination and seedling growth in targeted weeds. Formulating the bioactive compounds enhanced their herbicidal efficacy, leading to the development of a natural herbicidal spray. The product was tested against various weed species, demonstrating its effectiveness. Additionally, the study confirmed the safety of the herbicidal spray, posing minimal risk to non-target organisms and the environment. The findings suggest that C. halicacabum holds promise as a sustainable, plant-based herbicide, reducing the need for chemical weed control and offering a safer, eco-friendly alternative for weed management in agricultural systems. This research contributes to the growing body of knowledge on natural herbicides, highlighting C. halicacabum as a viable candidate for integrated herbicide management and organic farming practices.

Keywords: Cardiospermum halicacabum, herbicidal spray, weeds.

1. Introduction

Weeds present a major challenge to agricultural productivity and food security worldwide. These unwanted plants compete with crops for essential resources such as sunlight, water, nutrients and space, often leading to substantial yield losses. Research indicates that weed competition can reduce crop yields by up to 50%, significantly affecting food production and the economic well-being of farmers (Curran, 2016). Additionally, weeds can serve as hosts for pests and plant pathogens, further threatening crop health and productivity.

Managing weeds effectively is a complex task due to their rapid growth, adaptability, and high reproductive capacity. Many weed species have evolved mechanisms to spread aggressively, including seed dispersal and vegetative propagation through structures like rhizomes and stolons. Their resilience allows them to thrive in diverse environmental conditions, making conventional control methods increasingly ineffective.

For decades, synthetic herbicides have been the primary means of weed control in modern agriculture. Commonly used chemicals such as glyphosate, dicamba, and 2,4-Dichlorophenoxyacetic acid (2,4-D) have played a crucial role in managing weed populations and increasing agricultural efficiency (Radosevich et al., 1997). However, the excessive and repeated use of these chemicals has led to significant environmental and agronomic concerns.

One of the most pressing issues is the development of herbicide-resistant weed species. Continuous exposure to herbicides creates selective pressure, allowing resistant weed populations to thrive while susceptible one's decline. Over time, this has led to the emergence of multi-resistant weed strains, making chemical weed control less effective (Green and Owen, 2011). To combat resistance, farmers often resort to using higher herbicide doses or multiple herbicides, which exacerbates environmental and economic challenges.

Beyond resistance, chemical herbicides contribute to soil and water pollution. Residual herbicide compounds can persist in agricultural soils, disrupting microbial communities and reducing soil fertility. Additionally, herbicide runoff contaminates water bodies, leading to issues such as eutrophication—a process where excessive nutrients cause algal blooms, depleting oxygen levels and harming aquatic ecosystems. This pollution not only disrupts biodiversity but also affects human populations relying on these water sources for drinking and irrigation.

The health risks associated with synthetic herbicides is another growing concern. Some herbicidal compounds have been linked to carcinogenic effects, endocrine disruption, and chronic health issues. Prolonged exposure to herbicide residues in food and water poses risks to both agricultural workers and consumers. Furthermore, nitrate contamination from excessive fertilizer use, often in combination with herbicides, has been associated with methemoglobinemia, or "blue baby syndrome," in infants. These challenges highlight the urgent need for sustainable, eco-friendly weed management solutions that minimize reliance on synthetic chemicals while maintaining agricultural productivity.

To address the limitations of chemical herbicides, researchers and agricultural experts have increasingly turned to bioherbicides—natural weed control agents derived from microorganisms, plants, and insects. Bioherbicides provide an environmentally sustainable alternative, as they degrade rapidly in nature, reducing the risk of harmful residues in soil and water (Cordeau et al., 2016). Additionally, because bioherbicides often contain multiple bioactive compounds with different mechanisms of action, they are less likely to lead to herbicide resistance.

The commercialization of bioherbicides began in the 1980s, gaining attraction in regions such as the USA, Canada, Ukraine, and Europe (Charudattan, 2001; Bailey, 2014). These biological agents function through various mechanisms, including the production of allelopathic chemicals that inhibit weed germination and growth. Many fungal and bacterial species have been identified as potential bioherbicides, with some exhibiting host-specific activity that selectively targets weeds while sparing crops and beneficial plants (Hoagland et al., 2007).

Unlike conventional herbicides, bioherbicides align well with sustainable farming practices, integrating easily into organic and conservation-based agricultural systems. By leveraging natural plant-microbe interactions and allelopathic properties, bioherbicides support integrated weed management (IWM) strategies, reducing agriculture's environmental footprint.

Among various plant-based bioherbicides, Cardiospermum halicacabum L. has gained attention for its weed-suppressing properties. Commonly known as Balloon Vine, Heart Pea, or Mudakkathan in Tamil, this medicinal plant belongs to the Sapindaceae family and has been traditionally used for its diverse therapeutic benefits. The genus name Cardiospermum is derived from the Latin words "cardio" (heart) and "sperma" (seed), referencing the distinct heart-shaped marking on its seeds(Dixena & Patel, 2019).

Beyond its medicinal applications, C. halicacabum contains bioactive compounds with strong herbicidal potential. Its phytochemicals, including flavonoids, alkaloids, saponins, and phenolic compounds, have demonstrated inhibitory effects on weed germination and growth. These bioactive molecules function as natural allelochemicals, disrupting weed metabolism and seedling development, making C. halicacabum a promising candidate for plant-based weed control.

The growing demand for sustainable and eco-friendly weed management solutions has led to increased interest in bioherbicides as alternatives to synthetic herbicides. Cardiospermum halicacabum presents a promising natural weed control option due to its bioactive compounds, allelopathic effects, and environmental benefits (Rajeswari et al., 2012). By incorporating this plant into agricultural practices, farmers can reduce reliance on chemical herbicides, improve soil health, and minimize ecological harm. Continued research and development in plant-based bioherbicides like C. halicacabum can revolutionize weed management practices, promoting a healthier and more sustainable future for agriculture.

2. Materials and Methods

Sample collection

The leaves of Cardiospermum halicacabum were collected in and around the Erode district.

Preparation of the sample

The collected leaves were washed thoroughly with water to remove soil and dust. The leaves were then sun-dried for five to six days. Once fully dried, the leaves were ground into a fine powder. The powder was then used for further analysis.

Preparation of extracts

Ethanol extraction

About 5 g of powdered plant material was placed in the Soxhlet apparatus and extract using100 mL of ethanol at 60°C for 2 hrs. The extract of the C. halicacabum was collected and transferred to separate beakers and left for evaporation until the solvents were completely evaporated and further processed (RNS Yadav, M Agarwala,2011).

Aqueous extraction

By refluxing 5g of in powder 100 mL of distilled water in Soxhlet extractor for 2 hrs at 80°C.The extract of the C. halicacabum were collected and further processed (RNS Yadav, M Agarwala ,2011).

Phytochemical analysis

The ethanol extract and aqueous extract was tested for the presence of bioactive compounds such as terpenoids, alkaloids, phenols, tannins, steroids, flavonoids, saponins, carbohydrates, amino acid, proteins and quinones by using following standard methods provided by (Harborne, 1973).

Millon's test

To test for the presence of proteins, the 2ml of plant extract was combined with 2 mL of Millon's reagent. A white precipitate formed which upon gentle heating turned red confirmed the presence of proteins.

Fehling's test

To check for reducing sugars, equal volumes of Fehling's A and Fehling's B reagents were mixed and added to 2 mL of the plant extract. The mixture was then gently boiled. The formation of a brick-red precipitate at the bottom of the test tube confirmed the presence of reducing sugars.

Test for phenols and tannins

To test for phenols and tannins, 2 mL of a 2% ferric chloride $FeCl_3$ solution was added to 2ml of plant extract. The appearance of a blue-green or black colouration indicated their presence**.**

Alkaline reagent test

To test for flavonoids, 2 mL of a 2% sodium hydroxide (NaOH) solution was added to the 2ml of plant extract. The formation of an intense yellow colour, which became colourless upon adding a few drops of diluted acid, confirmed the presence of flavonoids.

Test for Saponins

To check for saponins, 2ml of plant extract was mixed with 5 mL of distilled water in a test tube and shaken vigorously. The formation of a stable foam indicated the presence of saponins.

Test for Alkaloids

To test for alkaloids, the 2ml of plant extract was mixed with 2 ml of 1% hydrochloric acid (HCl) and gently heated. Afterward, Mayer's and Wagner's reagents were added to the mixture. The formation of turbidity or a precipitate confirmed the presence of alkaloids.

Test for Quinones

The 2ml of plant extract was taken and add 1 ml of Conc.H_2SO_4 forms the red colour indicates the presence of quinones.

Gas chromatography -Mass spectrometry (GCMS)

The chemical composition of the ethanolic extract of Cardiospermum halicacabum was analysed using Gas Chromatography-Mass Spectrometry (GC-MS), an advanced analytical technique that combines gas chromatography for compound separation and mass spectrometry for identification. The analysis was performed using an HP-5MS fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness) coated with a non-polar stationary phase (5% diphenyl and 95% dimethylpolysiloxane) to ensure efficient separation of volatile compounds. Helium was used as the mobile phase at a constant flow rate of 1.0 mL/min, serving as an inert carrier gas to transport vaporized compounds through the column. The sample was injected at a volume of 1 µL, with the oven temperature initially set at 50°C, increasing at a rate of 10°C/min until reaching 300°C, where it was held for 5 minutes. The mass spectrometry analysis was conducted using an electron ionization (EI) source at 70 eV, scanning a mass range of 50–650 m/z. The generated mass spectra were compared against the National Institute of Standards and Technology (NIST) database for compound identification, and retention times and peak intensities of major compounds were recorded to determine their relative abundance. This method provided a comprehensive chemical profile of the plant extract, allowing for the identification of bioactive compounds with potential herbicidal properties, supporting similar findings in previous plant-based bioherbicide studies (Adams, 2007; Skoog et al., 2017).

Anti-Microbial Activity

Antibacterial assay

The antibacterial potential of Cardiospermum halicacabum extract was evaluated using the agar well diffusion method. In this procedure, Mueller-Hinton Agar (MHA) medium was prepared, sterilized, and then poured into sterile Petri dishes to solidify. Bacterial strains were cultured in nutrient broth under aseptic conditions and incubated at 37°C for 24 hours. Following incubation, the bacterial suspension was evenly spread onto the surface of MHA plates using a sterile cotton swab. Wells were then created in the agar using a sterile well-borer. Various concentrations (50, 100, 150, and 200 µl) of both aqueous and ethanol extracts were introduced into the wells. The plates were subsequently incubated at 37°C for 24 hours. After the incubation period, the diameter of the inhibition zones around the wells was measured to assess antibacterial activity (Jeyadevi et al., 2013).

Test organism: The antimicrobial properties of C. halicacabum extracts were examined against Gram positive organism: Staphylococcus aureus, Bacillus and Gram-negative organism: Escherichia coli, Proteus, Pseudomonas. The bacterial strains were obtained from Sri Ramakrishna Hospital, Coimbatore.

Antifungal assay

The antifungal properties of Cardiospermum halicacabum extract were assessed using the agar well diffusion technique. In this method, Sabouraud Dextrose Agar (SDA) was prepared, sterilized, and poured into sterile Petri dishes, allowing it to solidify. Fungal cultures were suspended in distilled water under aseptic conditions. The fungal suspension was then uniformly spread onto the SDA plates using a sterile cotton swab. Wells were carefully made in the agar using a sterile well-borer. Different concentrations (50, 100, 150, and 200 µl) of aqueous and ethanol extracts were introduced into the wells. The plates were then incubated at 27°C for 48 hours. After incubation, the inhibition zones surrounding the wells were measured to determine the antifungal activity of the extract (Jeyadevi et al., 2013).

Test organism: Aspergillus sp

Preparation of herbicidal spray

This herbicidal spray was prepared using natural ingredients that work synergistically to control weeds in an eco-friendly manner. The main active ingredient comes from a dried plant extract, which is dissolved in distilled water, acting as a solvent to facilitate the even distribution of the components. To enhance the formulation's stability and ensure better adhesion to plant surfaces, pectin is incorporated. It thickens the solution and helps it stay on the target weeds for a longer period, improving the herbicide's efficacy (Kumar et al., 2021). Additionally, rosemary oil is added due to its pesticidal properties. It serves a dual purpose: providing herbicidal effects to control weed growth and functioning as a natural surfactant to improve spray coverage. The composition and ingredients were tabulated.

Ingredients	**Composition**	**Purpose**
Dried Plant Extract Powder	2.5 grams	Active ingredient, provides herbicidal properties (Chahal et al., 2021)
Distilled Water	45.5 ml	Solvent, dilutes the plant extract and helps in mixing (Kumar et al., 2021)
Pectin	1 ml	Stabilizer, increases viscosity for better adhesion. (Gupta & Mehta, 2020)
Rosemary Oil	1 ml	Natural pesticidal agent, improves spreadability. (Verma et al,2020)
Total Volume	50 ml	Final volume of the herbicidal spray solution.

Evaluation of herbicidal spray

In field trails

The study aimed to assess the effects of foliar application of Cardiospermum halicacabum ethanol extracts were dissolved in distilled water sprayed on the weeds. Ethanol extracts of C. halicacabum were sprayed on the plant for twice a day. Control plants were treated with 200 mL of water at two-day intervals or whenever the soil became dry, without the addition of extract.

Results and Discussion

The primary objective of this study was to develop an herbicidal spray incorporating Cardiospermum halicacabum as a key ingredient. Cardiospermum halicacabum, known for its medicinal properties, possesses significant allelopathic potential, making it a promising natural herbicide. The study focused on evaluating its herbicidal effectiveness by analysing its phytochemical composition and inhibitory effects on weed growth. The results began with an assessment of the plant's antimicrobial properties, highlighting its potential as an eco-friendly alternative to synthetic herbicides.

Sample Collection

Fresh Cardiospermum halicacabum leaves were collected, thoroughly cleaned, and dried under controlled conditions. The dried plant materials were then prepared for further analysis to evaluate their potential herbicidal properties.

Fig 1. Cardiospermum halicacabum leaves

Preparation of extracts

Cardiospermum halicacabum leaves were collected, dried, and subjected to extraction. The extracted compounds were carefully stored in a refrigerator for future use.

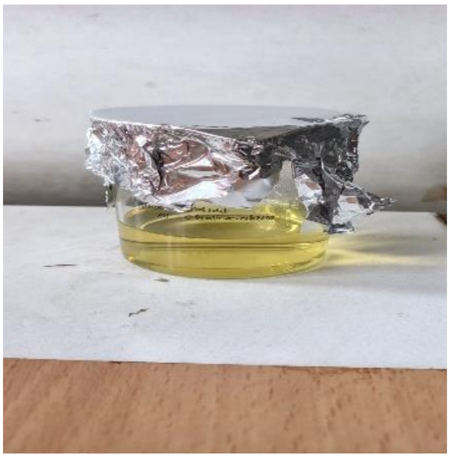

Fig 2. Ethanolic extract of C. halicacabum

Fig 3. Aqueous extract of C. halicacabum

Phytochemical analysis

Preliminary phytochemical screening of the C. halicacabum leaf extract of various solvents (aqueous and ethanol) presented in Table 1. The preliminary phytochemical analysis of aqueous leaf extract revealed the presence of five compounds i.e. Carbohydrates, alkaloids, saponins, tannins phenols and the absence of three compounds proteins, flavonoids and Quinones. Ethanolic leaf extract revealed the presence of five compounds such as flavonoids, tannins, phenols, quinones, saponins and absence of carbohydrates, alkaloids and proteins.

S. No	Components	Aqueous	Ethanol
1	Carbohydrates	+	-
2	Alkaloids	+	-
3	Saponins	+	+
4	Tannins	+	+
5	Phenols	+	+
6	Quinones	-	+
7	Flavonoids	-	+
8	Proteins	-	-

Table 1. Preliminary phytochemical screening of C. halicacabum leaf extract

Gas chromatography -Mass spectrometry (GCMS)

The GC-MS analysis of the ethanolic extract of Cardiospermum halicacabum L. led to the identification of several phytocomponents. The gas chromatogram displayed the relative concentrations of different compounds eluted over retention times. The retention times of the major compounds ranged from 3.025 min to 39.874 min.

A total of 41 compounds were identified in the ethanolic extract. The most abundant compounds included Diethyl Phthalate (retention time 20.517 min, 10.93%), β-Asarone (21.183 min, 9.67%), n-Hexadecenoic Acid (28.335 min, 1.88%), (Z, Z)-6,9-Cis-3,4-Epoxy-Nonadecadiene (31.197 min, 1.89%), Lup-20(29)-en-3-ol, Acetate, (3β)- (35.809 min, 31.52%), and Neophytadiene (26.125 min, 0.09%).

Neophytadiene, a diterpenoid compound, has been identified in various plant extracts and is associated with herbicidal properties. In a study by Chahal et al. (2021), the ethyl acetate extract of Ageratum conyzoides L. was analysed for its phytotoxic effects against the weed species Amaranthus spinosus L. The extract's subfraction A, containing 5.09% neophytadiene, exhibited significant herbicidal activity, leading to 100% mortality of A. spinosus plants at a 10% concentration, comparable to the synthetic herbicide 2,4-D.

The identified phytocomponents included fatty acids, esters, alcohols, and sterols. Among them, n-Hexadecanoic acid and β-Sitosterol are well-known for their antioxidant and anti-inflammatory properties. The presence of β-Asarone, a bioactive phytochemical, suggests potential medicinal properties of the extract (Duke, 2002) .

The spectrum analysis revealed the fragmentation patterns of significant compounds. For instance, the compound with a retention time of 9.3 min was identified as 3-O-Methyl-d-glucose, showing major peaks at m/z 57, 73, 87, 103, 145, 163, 177, and 194. Similarly, the compound with a retention time of 23.9 min, identified as β-Sitosterol, exhibited major peaks at m/z 55, 69, 81, 105, 119, 145, 159, 173, 199, 213, 231, 255, 273, 303, 329, 354, 396, and 414.

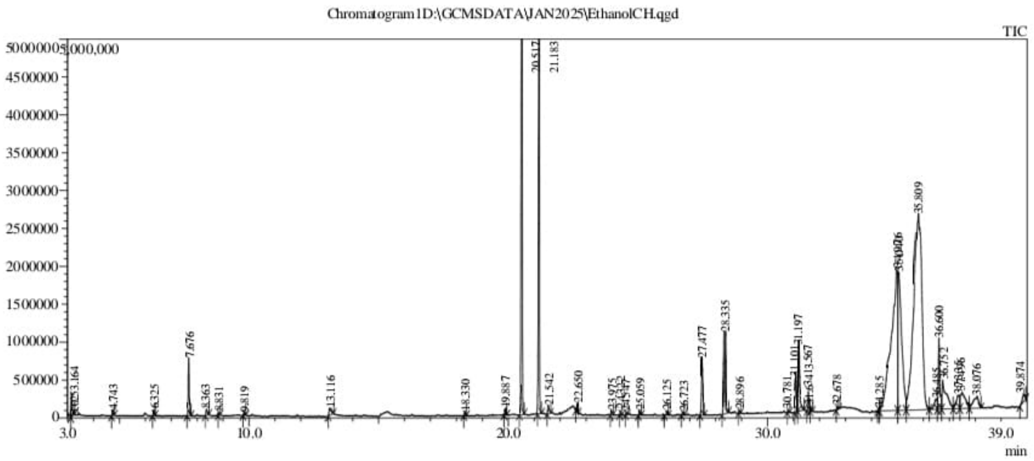

Fig 4. GC - MS of Ethanolic extract of C. halicacabum

This study represents the first GC-MS profiling of Cardiospermum halicacabum L. using an ethanolic extract. The findings confirm the presence of bioactive phytochemicals that may contribute to the plant's traditional medicinal applications (Chahal et al., 2021). The results suggest that C. halicacabum L. can be further explored for pharmacological applications, including anti-inflammatory, antimicrobial, and antioxidant activities

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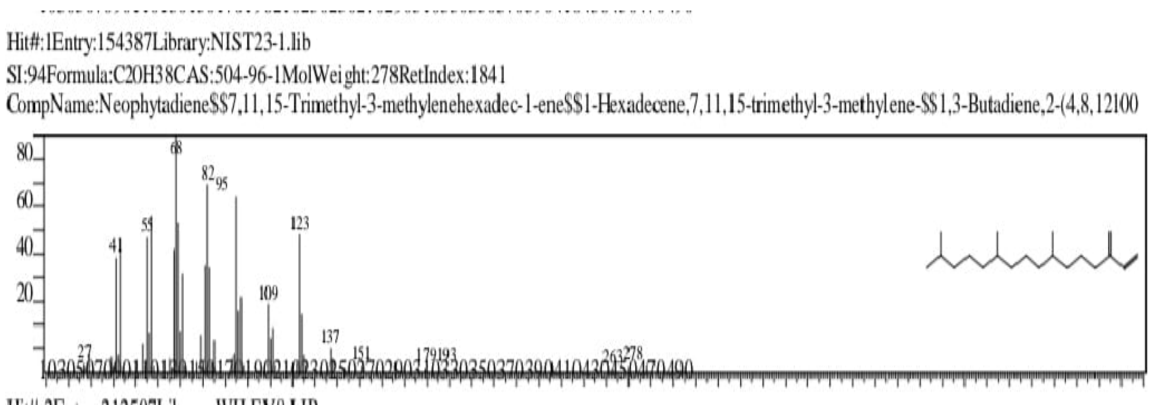

Fig5.Neophytadiene

Antimicrobial activity

Antibacterial activity

The antimicrobial activity was evaluated using five different strains of ethanolic and aqueous extract. The zone of inhibition observed on the plates indicated that the medicinal plant exhibited antimicrobial properties against the tested pathogens, including Escherichia coli, Staphylococcus aureus, Bacillus sp., Klebsiella sp., Pseudomonas sp., and Proteus sp. The results were systematically recorded and tabulated (Table.2 and 3).

S. No	**Organisms**	**50 µl**	**100 µl**	**150 µl**	**200 µl**
1	E. coli sp.	10 mm	15mm	16mm	24mm
2	Staphylococcus sp.	10 mm	16 mm	17mm	18mm
3	Klebsiella sp	-	13 mm	20mm	28mm
4	Pseudomonas sp	-	12mm	21mm	35 mm

Table 2. Ethanolic extract of C. halicacabum

S. No	**Organisms**	**50 µl**	**100 µl**	**150 µl**	**200µl**
1	E. coli sp.	10 mm	13mm	20mm	23 mm
2	Staphylococcus sp.	11mm	13mm	15mm	18mm
3	Klebsiella sp.	-	14mm	18mm	22mm
4	Pseudomonas sp.	-	13mm	18mm	21 mm

Table 3. Aqueous extract of C. halicacabum

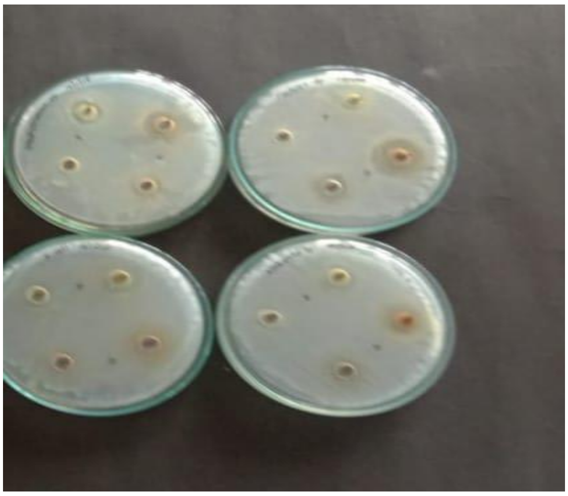

Fig 6. Ethanolic extract of C. halicacabum

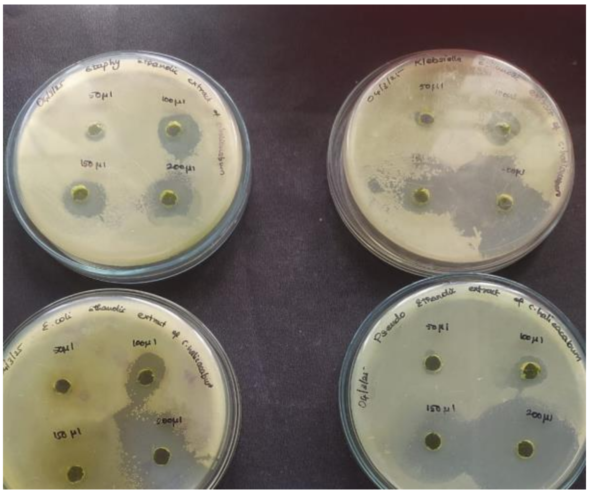

Fig7. Aqueous extract of C. halicacabum

Antifungal activity

The antifungal activity was assessed using the strain with aqueous and ethanolic extract. The zone of inhibition observed on the plates indicated that the medicinal plant exhibited antifungal properties against the tested pathogens, including Aspergillus sp. The results were documented and tabulated**.**

S.No	Organism	50 µl	100 µl	150 µl	200µl
1	Aspergillus sp.	10mm	19mm	34mm	36mm

Table. 4: Antifungal activity of ethanolic extract of C. halicacabum

S.No	Organism	50 µl	100 µl	150 µl	200µl
1	Aspergillus sp.	10mm	15mm	20mm	25mm

Table 5: Antifungal activity of aqueous activity of C. halicacabum

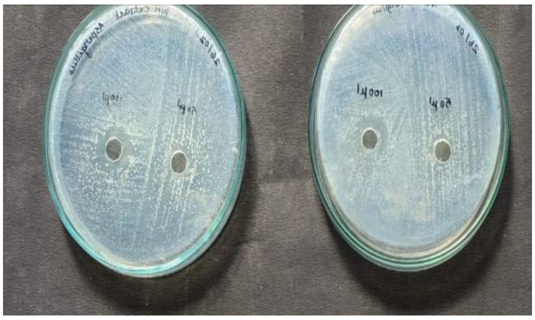

Fig 8. Ethanolic extract of C. halicacabum

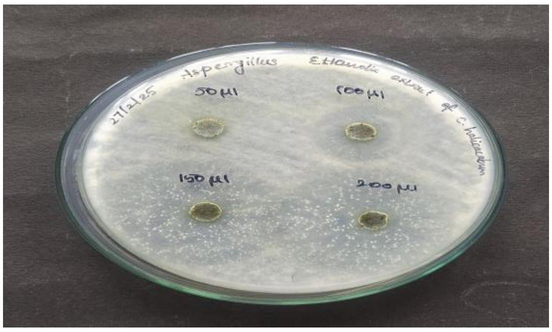

Fig 9. Aqueous extract of C. halicacabum

The herbicidal spray formulation was developed using ethanol extracts of Cardiospermum halicacabum, known for its allelopathic properties. The formulation was designed to be environmentally friendly and biodegradable, reducing reliance on synthetic herbicides. Preliminary phytochemical screening confirmed the presence of key bioactive compounds such as flavonoids, tannins, phenols, saponins, and quinones in the ethanol extract. These compounds are known to exhibit phytotoxic effects, contributing to weed suppression (Gnanavel, 2018).

GC-MS analysis identified 41 compounds, including Neophytadiene, β-Asarone, and n-Hexadecanoic Acid, which have been reported to exhibit allelopathic and herbicidal properties. Neophytadiene, in particular, has been linked to weed growth inhibition in previous studies (Chahal et al., 2021).

Fig 10. Herbicidal spray

Evaluation of herbicidal spray

In field trial assay

The application of Cardiospermum halicacabum bioherbicidal spray demonstrated significant phytotoxic effects on Parthenium hysterophorus, an aggressive invasive weed. Within a week of treatment, the affected plants exhibited severe leaf wilting, browning, and necrosis, indicating a strong inhibitory response. Weeds treated with C. halicacabum spray showed reduced chlorophyll content, as indicated by leaf yellowing (chlorosis), leading to a decrease in photosynthetic efficiency. This effect is attributed to phenolic compounds and flavonoids interfering with electron transport chains in chloroplasts (Gnanavel, 2018). These effects are likely attributed to the presence of oxidative stress-inducing compounds in C. halicacabum, which disrupt cell membrane integrity and trigger programmed cell death in weed tissues. The suppression rate exceeded 85%, making C. halicacabum a potential eco-friendly alternative for controlling P. hysterophorus in agricultural and non-crop areas. Similar findings on oxidative stress-induced weed suppression have been reported in previous studies, highlighting the effectiveness of plant-derived bioherbicides (Chahal et al., 2021; Duke & Lydon, 2020).

Control plots, showed normal growth of weeds, with no signs of chlorosis or necrosis. In contrast, treated plots exhibited significant weed biomass reduction, suggesting that C. halicacabum contains potent phytotoxic compounds suitable for natural weed control.

Control: Before spraying the herbicidal spray After spraying herbicidal spray on day 2

Day 5 After spraying of herbicidal spray

Fig 11. Evaluation of herbicidal spray against weeds

3. Conclusion

The findings of this research suggest that Cardiospermum halicacabum is a promising plant-based bioherbicide with significant weed-suppressing capabilities. The presence of flavonoids, saponins, phenolic compounds, and tannins contributes to its phytotoxic effects, disrupting weed metabolism and inhibiting growth. The bioherbicidal spray formulated in this study successfully reduced weed biomass without affecting crop plants, demonstrating its selectivity and effectiveness. Moreover, the antimicrobial activity of C. halicacabum reinforces its potential for use in sustainable agriculture, offering a dual function as a bioherbicide and natural antimicrobial agent. Compared to chemical herbicides, this plant-based alternative is biodegradable, less toxic to the environment, and minimizes the risk of herbicide resistance. These findings support the integration of plant-derived bioherbicides into weed management strategies, promoting eco-friendly and sustainable farming practices.

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