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Secondary metabolite profiling, TLC fingerprinting and antimicrobial assessment of *Parthenium hysterophorus* L. SCF extracts

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Abstract

Parthenium hysterophorus L. has become a global concern due to its invasive nature, rapid spread and allelopathic effects caused by volatile metabolites. This research investigates its potential benefits by extracting bioactive secondary metabolites using supercritical fluid extraction (SCFE) with CO_2, which is prized for its high density, low viscosity and excellent solvating properties allowing for efficient extraction with minimal residue. The crude extract was divided into fractions using solvents of increasing polarity n-hexane, chloroform and ethanol to obtain purified fractions. Qualitative phytochemical analysis and thin layer chromatography (TLC) identified key compounds, notably parthenin. The n-hexane fraction was abundant in terpenoids, saponins and tannins, indicating non-polar metabolites, while the ethanol fraction contained the highest levels of phenols (+++), flavonoids (++) and tannins (+++), confirming a wealth of polar constituents. T_9 showed distinct violet bands at Rf-0.6 in ethanol fractions (T_9) confirming the presence of parthenin. Antimicrobial testing revealed that the SCFE ethanol fraction demonstrated strong inhibition against *E. coli* (13.77 mm), *S. enterica* (14.40 mm) and *S. aureus* (14.55 mm) out performing Soxhlet extracts (5.17-5.33 mm) and nearing the activity of standard parthenin (15.13-22.57 mm). These results underscore SCFE as a superior green extraction method for isolating pharmacologically active compounds from *P. hysterophorus*, highlighting its potential as a source of medicinally valuable phytochemicals.

1. Introduction

Plant based products have been central to traditional medicine with systems like Ayurveda, traditional chinese medicine (TCM) and indigenous practices leveraging their therapeutic potential. TCM and Indian traditional medicine exemplify this heritage (Jaiswal *et al*., 2016; Patwardhan *et al*., 2005). According to Jaiswal *et al*. (2016), TCM documents 644 plant species and 8,980 medicinal materials, including 7,815 herbs. In India, the Indian Medicinal Plant Database lists 7,263 species, while the Traditional Knowledge Digital Library (TKDL) compiles 25,000 formulations, protected under intellectual property rights recognized by WIPO and EPO, yet remain scientifically underexplored (Mukherjee *et al*., 2012). The traditional medicine economy, valued at US$5.6 trillion in 2022, is projected to reach US$8.5 trillion by 2027. Scientific validation ensures safety, while indigenous knowledge remains vital but endangered. Nearly half of modern drugs derive from natural products with many traditional formulations untapped (Kuruvilla *et al*., 2024). India's medicinal systems-including Ayurveda, Siddha, Unani, Folk and Tribal Medicine, Homeopathy and Yoga-emphasize holistic balance of body, mind and environment with Ayurveda being the most widely practiced (Elahee *et al*., 2019; Patwardhan and Mashelkar, 2009). The demand for medicinal plants, rich in alkaloids, flavonoids, tannins, steroids and terpenoids, underscores their pharmaceutical value. A quarter of modern drugs are plant derived, including aspirin, morphine and reserpine, as well as contraceptive pills from yam, artemisinin for malaria and childhood cancer treatments from rosy periwinkle (Kuruvilla *et al*., 2024). Key bioactives such as curcumin, withaferin A and kutkoside show strong therapeutic effects, while formulations like gugulipid and boswellia extracts are used to treat dyslipidemia and inflammation. The integration of traditional knowledge with modern research drives drug discovery, exemplified by polyherbal products such as Artrex for arthritis (Patwardhan *et al*., 2005).

Parthenium hysterophorus L., of the Asteraceae family, occurs in tropical and subtropical regions. This genus includes trees, herbs and shrubs with white flowers. As an invasive species, it contains

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parthenin, which provides resistance to abiotic stresses and aids in bioactive compound production (Jambamma *et al*., 2025). Invasive plants infiltrating natural ecosystems pose significant risk to biodiversity. *P. hysterophorus* suppresses nearby vegetation growth, reduces agricultural productivity and causes health issues like contact dermatitis and respiratory allergies (Morin *et al*., 2009; Pablos *et al*., 2017). Although, this plant has harmful effects, studies worldwide have highlighted its beneficial uses. Solvent extracts from this weed have shown promise as a natural source for anticancer, anti-HIV, antimicrobial and antifungal treatments (Dhiman *et al*., 2018; Jaiswal *et al*., 2022; Kumar *et al*., 2013). It has shown antioxidant activity facilitated development of silver nanoparticles (Sivakumar *et al*., 2021) and served as a source of bioactive compounds like phenols, flavonoids, saponins, terpenes, tannins and steroids. It is used in traditional medicine (Algfri *et al*., 2022; Jaiswal *et al*., 2022; Kumar *et al*., 2013a), as feedstock for industrial treatment of metal and dye wastewater (Dipesh and Rajiv, 2018; Lata *et al*., 2008) and for biogas production. This study aimed to extract heat sensitive secondary metabolites using a supercritical fluid extractor at low temperatures to minimize solvent residue. The extract was then fractionated with n-hexane, chloroform and ethanol to perform qualitative phytochemical analysis, quantitatively identify parthenin using TLC and evaluate antimicrobial sensitivity against common pathogens.

2. Materials and Methods

2.1 Collection of plant material and preparation of extracts

The non-flowering vegetative stage of *P. hysterophorus* was collected from local waste and barren lands located at 16.2160° N, 77.3566°E. The species was authenticated by the Department of Horticulture, Agricultural College, Aswaraopet (ACA/PJTSAU/HORT/Weed/Parthenium/03) and a herbarium specimen was issued by Dr. I. V. Srinivas Reddy, Professor, PJTAU, Hyderabad (Jambamma *et al*., 2024). Fresh samples of *P. hysterophorus* roots were removed and the aerial parts (leaves and stems) were thoroughly washed with running and double distilled water, then dried in a dehumidified air dryer at 40°C and 15% relative humidity. The dried material was finely ground, passed through a 250 µm sieve and stored in a freezer for subsequent extraction and analysis.

2.2 Chemicals and reagents

Analytical grade reagents were used throughout the study. Mueller–Hinton agar (MHA), dimethyl sulfoxide (DMSO), Luria Bertani broth (Miller) and standards were procured from HiMedia Laboratories (Mumbai, India). The solvents and reagents, including Ellagic acid (standard), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), ferric chloride ($FeCl_4$) solution (1%), lead acetate (10% solution), Mayer's reagent, Valinine reagent, hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_2), chloroform, ethanol (95%) and n-hexane, were purchased from M/S. Sigma Aldrich Chemicals (St. Louis, MO, USA) and silica gelcoated TLC plates (Merck, Darmstadt, Germany) were procured from Merck India. All other chemicals and reagents used in the study were of analytical and molecular grade.

2.2.1 Supercritical fluid extraction

Supercritical fluid extraction (SCFE) is done using the Waters Thar SFE 500 system. The SCFE process used CO_2 at a flow rate of 2 g/min with ethanol as a co-solvent at 20 g/min. A 100 g powdered sample was subjected to extraction under supercritical conditions.This process was conducted at three different pressure levels (100, 150 and 200 bar) and three temperatures (40, 50 and 60°C), resulting in a total of nine SCFE extracts (T_1-T_9). The pressure of 100 bar was chosen because it is just above the critical pressure of CO_2, which is 73 bar. This decision was based on earlier studies (Liza *et al*., 2010; Wang *et al*., 2008). To avoid damaging heat sensitive compounds, the highest extraction temperature was set at 60°C, as mentioned in the study by Cossuta *et al*. (2008). The extraction time was kept the same at 120 min for all tests.

2.2.2 Soxhlet extraction

Bioactive compounds from *P. hysterophorus* powder were extracted using a Soxhlet apparatus (SOCS-PLUS, Pelican SCS-08) with ethanol. About 100 g of dried powder was placed in the extractor thimble and extracted with 300-350 ml ethanol for 120 min at 85°C. The obtained extract (T_{10}) was then concentrated by removing the solvent under reduced pressure using a rotary vacuum evaporator (Superfit Rotavap PBU-6D) (Martinez *et al*., 2010).

2.3 Sequential fractionation of *P. hysterophorus* SCFE crude drug

The SCFE extracts (T_1-T_9) and control (T_{10}) was sequentially fractionated with solvents of increasing polarity (1:1,v/v) using centrifugal vials. The mixtures were centrifuged (5810R centrifuge, Eppendorf, Hamburg, Germany) at 18,500 × g for 10 min at 24°C, following modified protocols (Cho *et al*., 2010; Dickson, 1979; Kumar *et al*., 2014). The resulting solvent fractions were evaporated to concentration and stored in the dark at 20°C until use (Figure 1a: n-hexane fraction, Figure 1b: chloroform fraction and Figure 1c: ethanol fraction).

2.4 Phytochemical profiling of SCFE fractionated extracts

The qualitative chemical analysis of plant metabolites in the extracts was performed to identify major phytoconstituents such as total phenols, total flavonoids, alkaloids, tannins, terpenoids and saponins present in the *P. hysterophorus* aerial part extracts obtained from SCFE n-hexane, chloroform and ethanol fractions, following standard protocols (Harborne, 1973; Trease and Evans, 1989).

2.5 Thin layer chromatography (TLC) finger printing of SCFE fractionated extracts

Thin layer chromatography (TLC) was utilized to detect phyto-constituents, with a focus on parthenin, in solvent-fractionated extracts (n-hexane, chloroform, and ethanol; T_1-T_9) as per the methods of Hildebert and Sabine (1996) and Jaiswal *et al*. (2022). Silica gel G TLC plates (Merck, Darmstadt, Germany) were dried, activated at 110°C for 90 min and then allowed to cool to room temperature. Extracts with different polarities were dissolved in suitable solvents and applied 1 cm above the baseline using fine capillaries. Among the tested systems, a chloroform-ethanol mixture (3:1,v/v) was found to be the optimal mobile phase, enabling solvent migration up to 80% of the plate. Distinct bands were observed, and Rf values were determined as Rf = distance traveled by solute/distance traveled by solvent. Plates treated with vanillin reagent and viewed under UV light (254 nm) revealed violet bands, indicating the presence of parthenin. A notable violet spot at Rf 0.6 confirmed parthenin, which was further corroborated by its HPLC retention time of 5.8 min (Hernandez *et al*., 2011).

Figure 1: Sequential fractionations of SCFE parthenium extracts into non-polar and polar solvents using: a.n-hexane, b. chloroform and c. ethanol.

2.6 Antimicrobial activity

The SCF Eextracted *P. hysterophorus* ethanol fraction, enriched with phenolic and flavonoid compounds, exhibited notable antimicrobial activity. Two controls ethanol Soxhlet extract and standard parthenin-were used for comparison. The antimicrobial activity was evaluated using the Disc diffusion method (Shrimali *et al.,* 2001) against foodborne pathogens *Escherichia coli (E. coli)*, *Salmonella enterica S. enterica)* and *Staphylococcus aureus (S. aureus)* obtained from MTCC, Chandigarh. Disc preparation: Whatman No. 1 filter paper discs (6 mm) were sterilized at 121°C for 15 min. Stock solutions (100 µg ml^{-1}) were prepared by dissolving 0.8 g extract in 2 ml DMSO (Bukar *et al*., 2010). Sensitivity test: Cultures were maintained on nutrient agar and subcultured on broth. The Disc agar diffusion method (Bauer *et al*., 1996; Cakir *et al*., 2004) was employed. Inoculated MH plates (10 ml medium + 4 ml inoculum) received extract soaked discs, refrigerated for 2 h, then incubated at 37°C for 24 h. Antimicrobial activity was assessed by measuring the zone of inhibition (mm) around the discs (Albouchi *et al*., 2013).

2.7 Statistical analysis

All experiments were performed in triplicate, and results were reported as mean ± standard error (SE). A two-way ANOVA without replication was used to assess the effects of microbial strain and treatment type, with significance set at $p = 0.05$. Data analysis was performed using Microsoft Excel (2010).

3. Results

3.1 Qualitative phytochemical screening of SFCE solvent fractionated *P. hysterophorus* extracts

The SCFE crude extract (T_1 to T_9 and T_{10}) from vegetative stage *P. hysterophorus* powder were subjected to sequential solvent fractionation using solvents of increasing polarity: n-hexane, chloroform and ethanol. All fractions were qualitatively analyzed detailed in Section 2.3 above.

The qualitative phytochemical screening of *P. hysterophorus* extracts showed distinct distributions of secondary metabolites across different solvent systems n-hexane, chloroform and ethanol. The colour reactions and screening results presented in Table 1 confirm the presence of various secondary metabolites in these extracts.The n-hexane extracts contained phenols (T_6, T_9), flavonoids (T_1, T_5, T_8), terpenoids (T_2, T_3, T_7, T_8, T_{10}), saponins (T_2, T_6, T_8, T_9), tannins (T_3, T_5, T_6, T_7, T_9, T_{10}) and alkaloids (T_1, T_4, T_5, T_8, T_{10}) with higher levels of terpenoids, saponins and tannins, indicating dominance of non-polar metabolites. Chloroform extracts showed phenols (T_1, T_6, T_7, T_9), flavonoids (T_8, T_{10}), terpenoids (T_3, T_4, T_5, T_8, T_{10}), saponins (T_1, T_6, T_7, T_9), tannins (T_1, T_6, T_7, T_9) and alkaloids (T_5) with terpenoids and tannins as major constituents.

Ethanol extracts confirmed phenols (T_7, T_9, T_{10}), flavonoids (T_1, T_3, T_4, T_5, T_8, T_9), terpenoids (T_2, T_4, T_6, T_8), saponins (T_2, T_3, T_4), tannins (T_1, T_7, T_9, T_{10}) and alkaloids (T_3, T_5, T_8, T_9). The T_9 ethanol fraction showed the highest intensity for phenols (+++), flavonoids (++) and tannins (+++), indicating the richest concentration of polar phytochemicals. Overall, n-hexane and chloroform fractions were rich in non-polar metabolites, while ethanol efficiently extracted polar compounds with T_9 being the most concentrated fraction.

3.2 TLC fingerprint based detection of parthenin in solvent fractionated extracts under UV illumination

TLC remains a primary method for identifying natural compounds listed in pharmacopoeias, providing distinct herbal fingerprints (Algfri *et al*., 2022). The TLC fingerprint analysis in this study supported the qualitative phytochemical findings and enabled the detection and quantification of parthenin in solvent fractionated *P. hysterophorus* extracts (n-hexane, chloroform and ethanol; T_1-T_{10}). Retention factor (Rf) values were determined for each fraction using a standard parthenin reference compound.

Table 1: Identification of major phytoconstituents in n-hexane, chloroform and ethanol fractions from the SCFE extracts, T_1 to T_9 and T_{10} of *P. hysterophorus* powder

Test type	Type of solvent	SCFE extract solvent fractionates									
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Total phenols ellagic acid test	n-Hexane	-	-	-	-	-	++	-	-	++	-
	Chloroform	+	-	-	-	-	+	+	-	+	-
	Ethanol	-	-	-	-	-	-	++	-	+++	++
Total flavonoids ferric chloride test	n-Hexane	+	-	-	-	+	-	-	+	-	-
	Chloroform	-	-	-	-	-	-	-	+	-	+
	Ethanol	+	-	+	+	+	+	-	+	+++	-
Alkaloids mayears reagents	n-Hexane	+	-	-	+	+	-	-	++	-	+
	Chloroform	-	-	-	-	+	-	-	-	-	-
	Ethanol	-	-	+	-	+	+	-	+	++	-
Tannins lead acetate	n-Hexane	-	-	+	-	+	+++	++	-	+++	+
	Chloroform	+	-	-	-	-	+	+	-	+	-
	Ethanol	+	-	-	-	-	-	++	-	++	+++
Tarpenoids salkowski test	n-Hexane	-	+	+	-	-	-	++	+++	-	+
	Chloroform	-	-	+	+	++	-	-	+	-	++
	Ethanol	-	++	-	+	-	+	-	+	-	-
Saponin efoam test	n-Hexane	-	+	-	-	+	++	-	+++	-	-
	Chloroform	+	-	-	-	-	+	+	-	+	-
	Ethanol	-	+	+	+	-	-	-	-	-	-

- represents absent, +, ++ and +++ represents light, moderate and dark color fractions

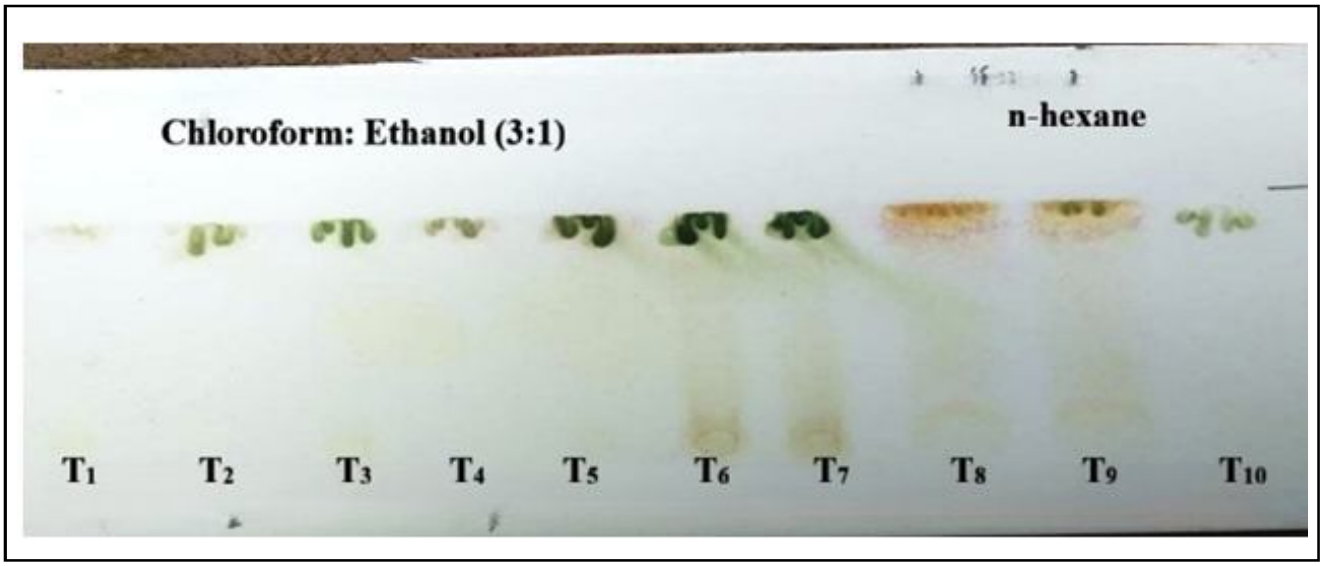

Figure 2a: Identification of parthenin compound in n-hexane fractionates of the SCFE extracts of *P. hysterophorus* using TLC technique.

3.2.1 TLC analysis of *P. hysterophorus* n-hexane fraction

Figure 2a depicts the separation of SCFE n-hexane fractions (T_1-T_9 and T_{10}) on a TLC plate, utilizing a mobile phase of chloroform and ethanol in a 3:1 ratio. Under UV light at 254 nm (Figure 2b), no distinct violet spots were observed, indicating the absence of parthenin in the n-hexane fractions. The reference parthenin compound had an Rf value of approximately 0.6. However, dark violet spots appeared near the top of the plate with fractions T_8 and T_9 displaying prominent violet-blue bands close to the upper edge, suggesting the presence of bioactive compounds with high Rf values.

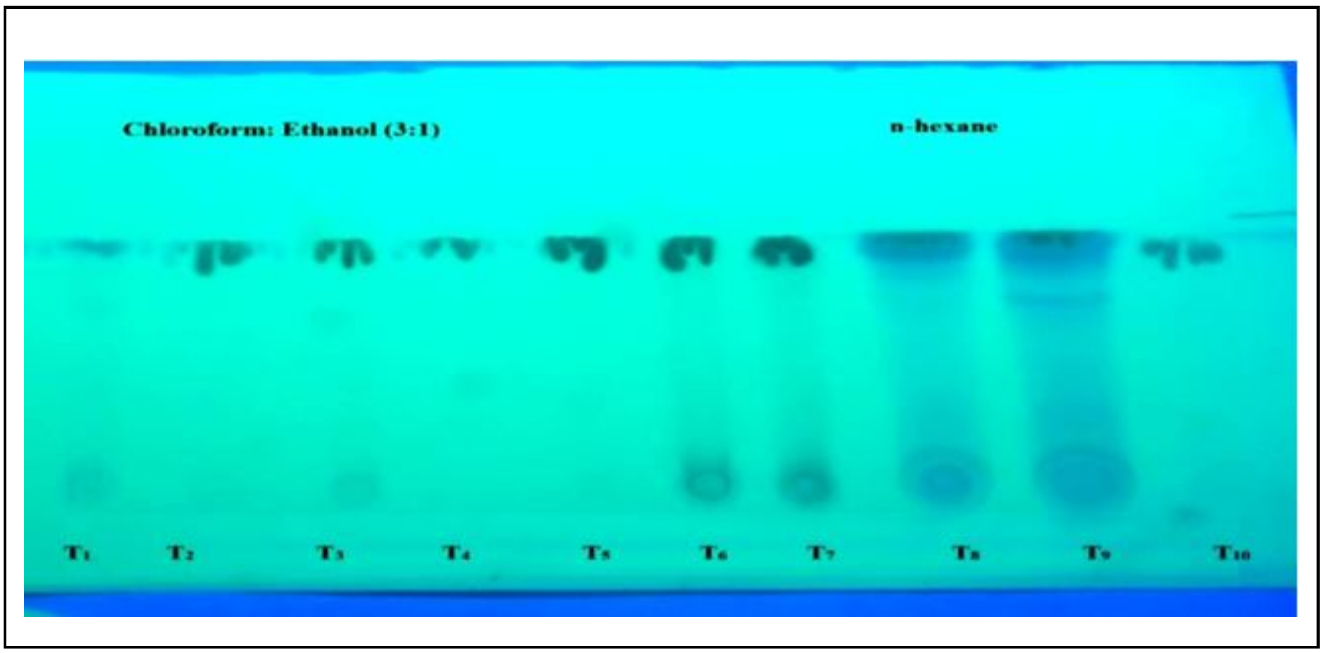

Figure 2b: Parthenin compounds detected in n-hexane fractionates of the SCFE extracts of *P. hysterophorus* at UV light of 254 nm after spraying the vallinin reagent.

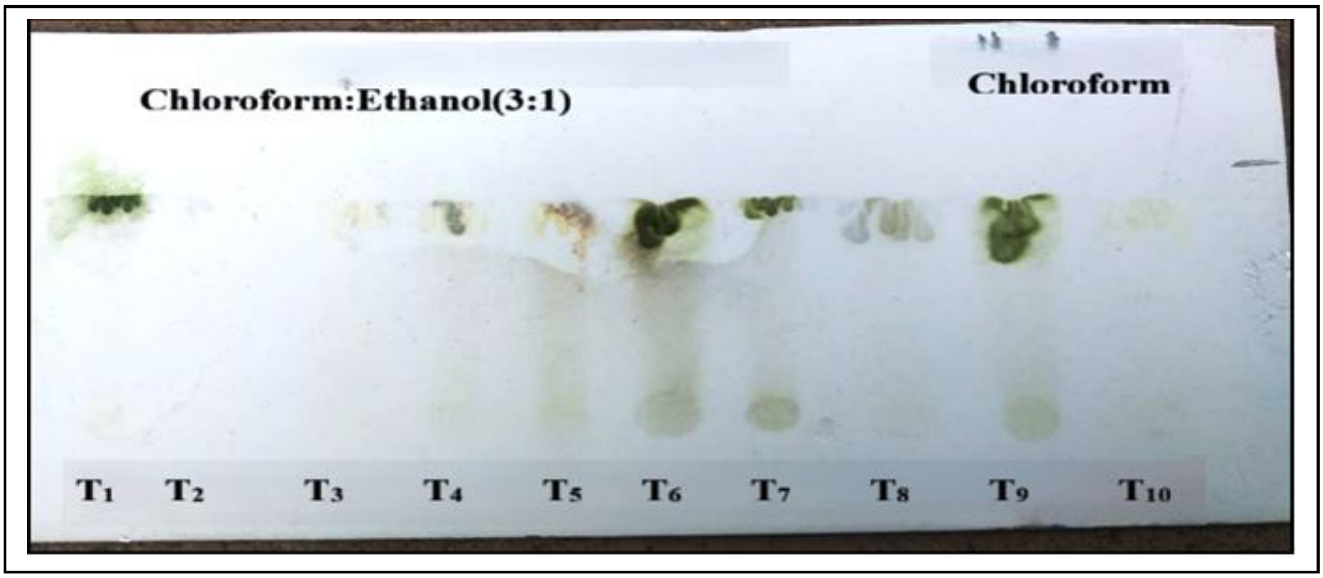

Figure 3a: Identification of parthenin compound in chloroform fractionates of the SCFE extracts of *P. hysterophorus*using TLC technique.

3.2.2 TLC analysis of *P. hysterophorus* chloroform fraction

Figure 3a illustrates a TLC plate with ten lanes (T_1-T_{10}) that represent the SCFE chloroform fractions, developed using a solvent system of chloroform and ethanol in a 3:1 ratio. When exposed to UV light at 254 nm, a band with an Rf value of 0.6 matched the standard parthenin sample. However, bands in fractions T_4 and T_5 appeared at higher Rf values, indicating the presence of secondary metabolites with increased mobility due to variations in polarity and interactions with the stationary phase. The emergence of distinct dark violet spots near the top of the plate further confirmed these secondary metabolites and demonstrated the absence of parthenin in the chloroform fractions.

3.2.3 TLC analysis of *P. hysterophorus* ethanol fraction

Figure 4a shows the TLC profile of SCFE ethanol fractions (T_1-$T_9$and T_{10}) developed with a chloroform-ethanol (3:1) solvent system. When exposed to UV light at 254 nm, several violet bands emerged, corresponding to parthenin with an approximate Rf value of 0.6. These distinctive bands were notably present in fractions T_1, T_2, T_5, T_6, T_7, T_8, T_9 and T_{10}, indicating the presence of parthenin and related bioactive compounds in the ethanol extract fractions as indicated in Figure 4a .

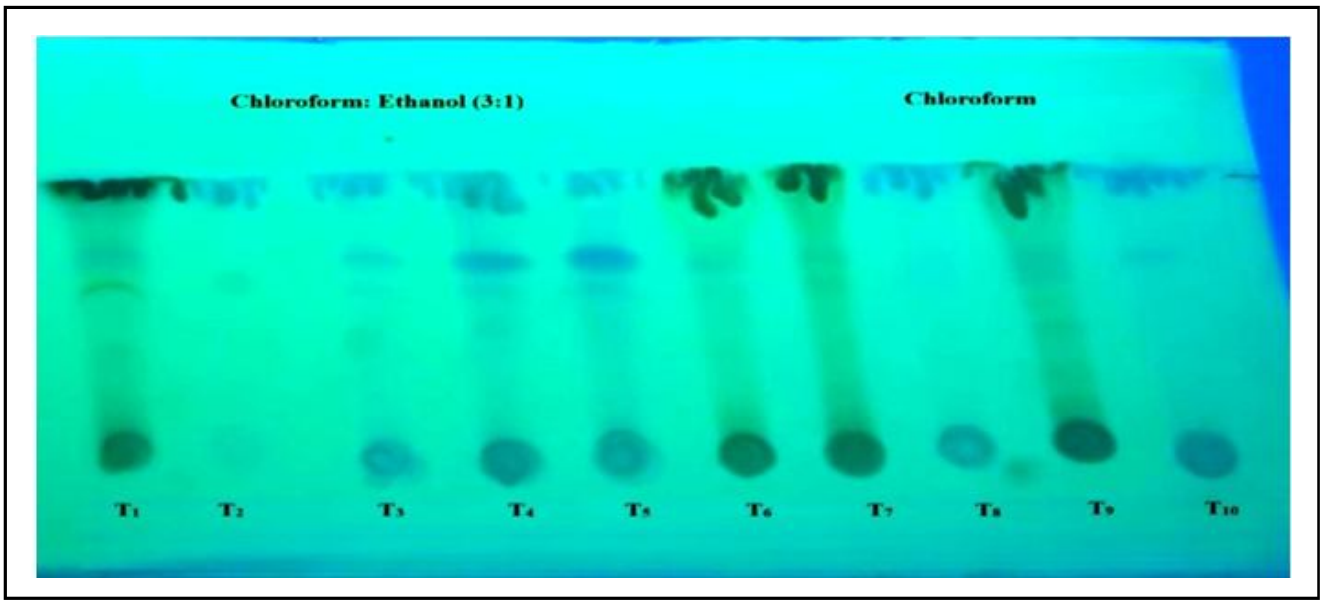

Figure 3b: Parthenin compounds detected in chloroform fractionates of the SCFE extracts of *P. hysterophorus* at UV light of 254 nm after spraying the vallinin reagent.

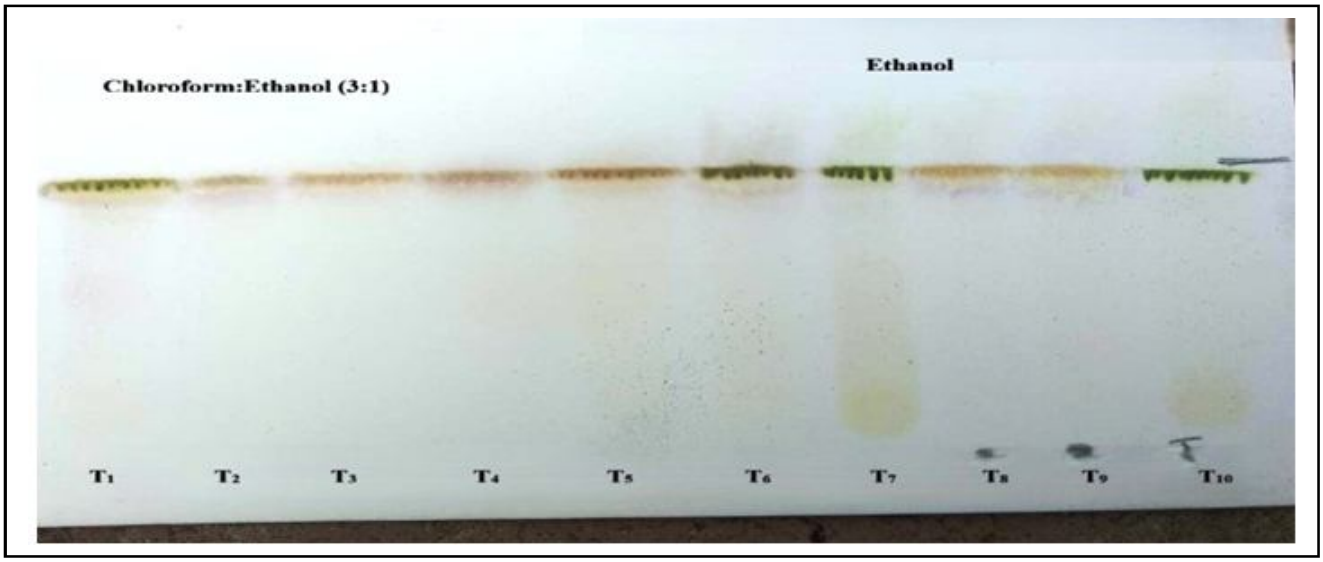

Figure 4a: Identification of parthenin compound in ethanol fractionates of the SCFE extracts of *P. hysterophorus* using TLC technique.

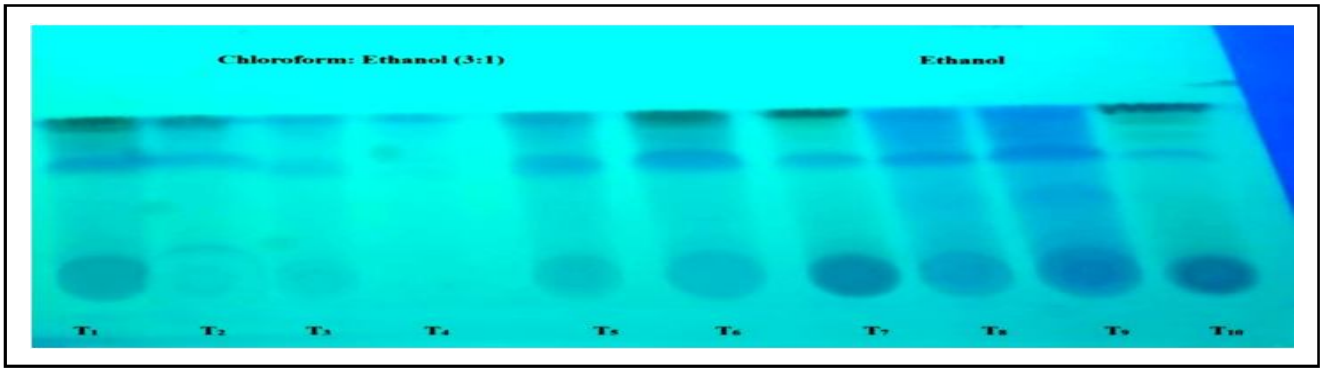

Figure 4b: Parthenin compounds detected in ethanol fractionates of the SCFE extracts of *P. hysterophorus* at UV light of 254 nm after spraying the vallinin reagent.

3.3 *In vitro* antimicrobial efficacy of SCFE ethanol fraction from *P. hysterophorus* against common pathogens

The antimicrobial effectiveness of ethanol fractions from *P. hysterophorus*, extracted using supercritical fluid extraction (SCFE), was assessed against *E.coli*, *S.enterica* and *S. aureus*, as described in Section 2.5. Comparative studies were conducted using Soxhlet ethanol extract and standard parthenin as benchmarks. The findings in Table 2 indicated that the inhibition zones ranged from 5.17 ± 0.12 to 21.23 ± 0.45 mm for *E. coli*, 5.33 ± 0.15 to 15.13 ± 0.06 mm for *S. enterica* and 5.12 ± 0.09 to 22.57 ± 0.06 mm for *S. aureus*. For *E. coli*, the ethanol fraction (T_9) achieved a maximum inhibition zone of 13.77 mm, while the Soxhlet extract exhibited minimal activity (5.17 mm). The standard parthenin showed the highest inhibition (21.23 mm) (Figures 5). Similar patterns were observed, Figures 6, for *S. enterica*, where the SCFE extract produced a zone of 14.40 mm, comparable to parthenin (15.13 mm) and significantly greater than the Soxhlet extract (5.33 mm).

Against *S.aureus*, the SCFE ethanol fraction, T_9, recorded a maximum inhibition of 14.55 ± 0.15 mm, whereas the Soxhlet extract had the lowest (5.12 ± 0.09 mm). The standard parthenin demonstrated significantly higher activity (22.57 ± 0.06 mm) as shown in Figure 7. Overall, the SCFE ethanol fractions exhibited notable antimicrobial potential, surpassing conventional Soxhlet extracts and showing activity levels close to that of standard parthenin.

From ANOVA Table 3, microbial strain did not exert a significant effect on inhibition zones ($F = 0.87$, $p = 0.485$). Conversely, treatment type had a highly significant influence ($F = 28.81$, $p = 0.004$), demonstrating substantial differences in antimicrobial activity among the Soxhlet extract, SCFE extract and standard parthenin.

Table 2: *In vitro* antimicrobial effects of supercritical fluid extracted *P. hysterophorus* parthenin test drug

Name of organism	Zone of inhibition (ZOI, mm)		
	Soxhlet extract (T_{10})	SCFE ethanol extracts (T_9)	Standard parthenin
E. coli	5.17 ± 0.12	13.77 ± 0.45	21.23 ± 0.45
S. enterica	5.33 ± 0.15	14.40 ± 0.36	15.13 ± 0.06
S. aureus	5.12 ± 0.09	14.55 ± 0.15	22.57 ± 0.06

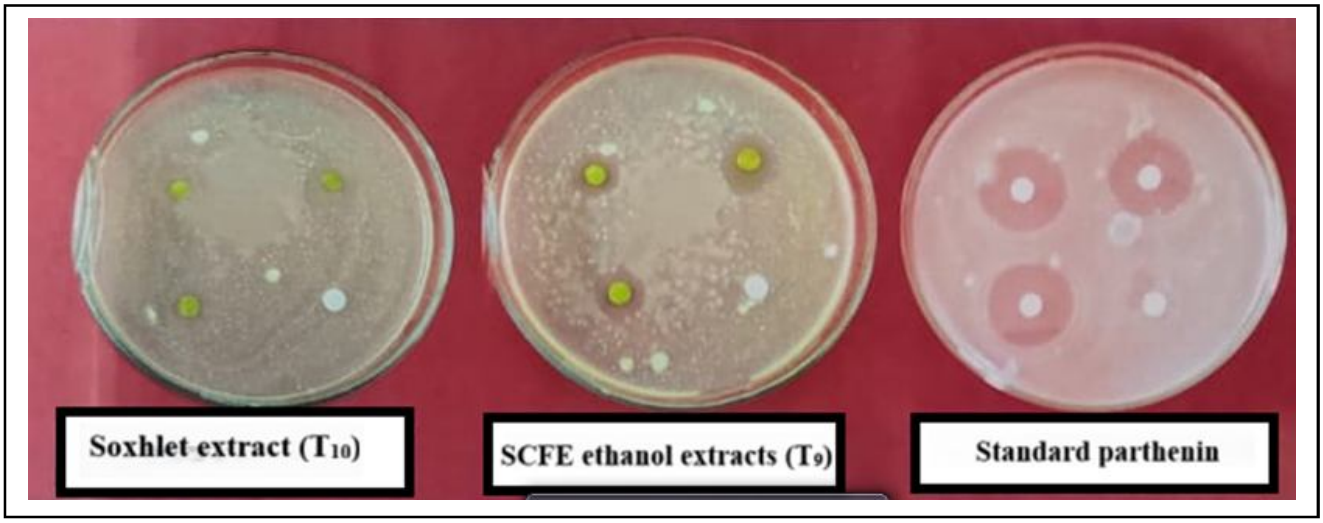

Figure 5: Effect of SCFE extracted *P. hysterophorus* powder parthenin test drug against *E. coli.*

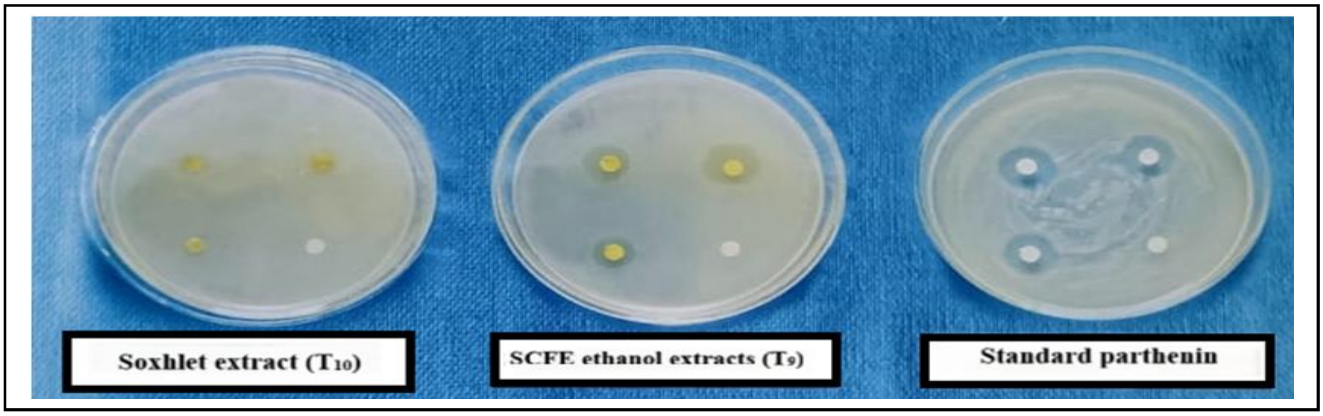

Figure 6: Effect of SCFE extracted *P. hysterophorus* powder parthenin test drug against *S. enterica.*

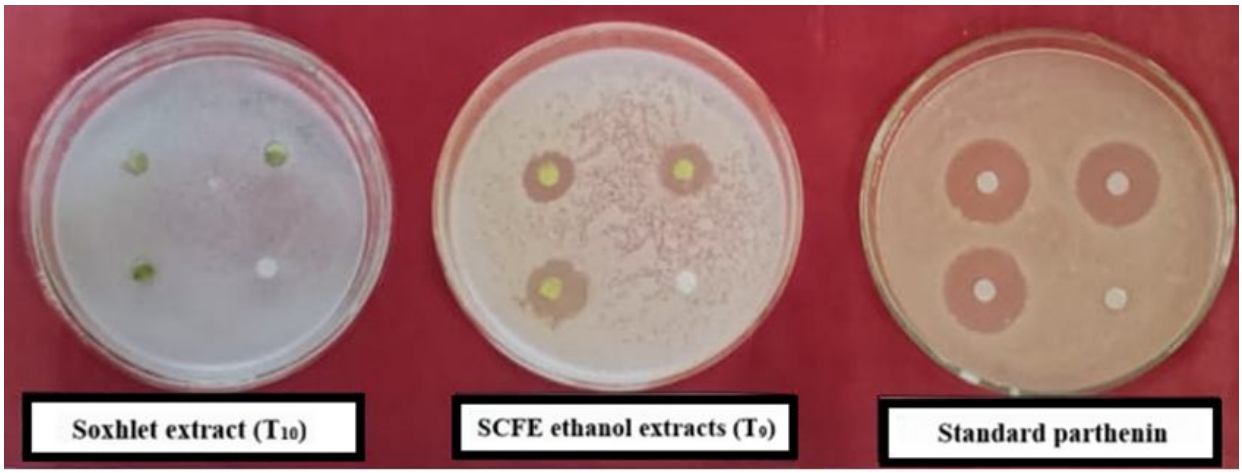

Figure 7: Effect of SCFE extracted *P. hysterophorus* powder parthenin test drug against *S. aureus.*

Table 3: Two-factor ANOVA (without replication) for the effects of microbial strain and treatment type on zone of inhibition zones

Source of variation	S S	df	MS	F	p-value	F_{crit}
Rows (Microbial strains)	9.6606	2	4.8303	0.8719	0.4849	6.9442
Columns (Treatments)	319.2145	2	159.6072	28.8111	0.0042	6.9442
Error	22.15913	4	5.5398			
Total	351.0342	8				

4. Discussion

In India, traditional medical systems account for about 70% of the pharmaceutical market, reflecting a strong heritage in plant based medicine (Meena and Indiragandhi, 2025). Globally, the demand for medicinal plants continues to rise with the market projected to grow from US$5.6 trillion in 2022 to US$8.5 trillion by 2027 (Kuruvilla *et al*., 2024). Phytochemicals natural compounds found in medicinal plants exert significant physiological effects in humans and animals and serve as key precursors in modern drug synthesis. Major groups such as alkaloids, tannins, flavonoids, terpenoids and anthocyanins have demonstrated potent *in vitro* antibacterial, antioxidant and anti-inflammatory activities (Meena and Indiragandhi, 2025; Sumaiya *et al*., 2025).

This study explored the phytochemical characteristics and medicinal potential of *P. hysterophorus*, a widely spread invasive species recognized for its allelochemicals like parthenin, which aid in its ecological dominance. Traditional eradication techniques, including biological, mechanical and chemical methods, have been both expensive and ineffective. However, the plant's ethnomedicinal significance in regions such as India, Pakistan and parts of Africa where it is utilized for its antibacterial, antifungal, anticancer and antioxidant properties presents a sustainable alternative (Patel, 2011).

The innovation of this research lies in selecting *P. hysterophorus* during its non-pollinated vegetative phase to minimize inhalation risks and boost bioactive yield. Conventional extraction methods like maceration, distillation and Soxhlet are slow, yield low amounts and are susceptible to solvent or heat degradation. To overcome these issues, supercritical fluid extraction (SCFE) using CO_2 and ethanol was applied under low temperature, highpressure conditions, allowing for quicker and more efficient extraction while preserving compound stability (Nejia *et al*., 2013).

A comparative study examined essential oil extraction from clove buds using supercritical CO_2 (SCFE), hydrodistillation (HD), steam distillation (SD) and Soxhlet extraction (SE) (Guan *et al*., 2007). SFE produced 19.56% essential oil with 58.77% eugenol, outperforming HD (11.5%, 50.3%), SD (10.1%, 61.2%) and SE (41.8%, 30.8%). Its mild operating conditions preserved thermolabile compounds while enhancing yield. Likewise, SC-CO_2 efficiently extracted volatile compounds such as luteolin from spearmint (Bimakar *et al*., 2011). The increased pressure and temperature during extraction enhance CO_2 density and solvent strength, improving the recovery of phenolic, flavonoid and alkaloid compounds (Calista *et al*., 2016; Nejia *et al*., 2013). Thus, SFE emerges as a clean, efficient and sustainable alternative to conventional extraction techniques.

The vegetative parts of *P. hysterophorus* are rich in both primary and secondary metabolites. Supercritical fluid extraction (SCFE) proved particularly effective in isolating heatsensitive bioactive compounds, yielding volatile constituents with potential pharmaceutical applications for chronic diseases such as cancer. The SCFE crude extract was subsequently subjected to solvent fractionation using solvents of increasing polarity to enable the selective isolation and concentration of bioactive components. This approach enhanced the antioxidant, antimicrobial and anticancer properties of the extracts.

Phytochemical screening of various solvent extracts confirmed the presence of diverse secondary metabolites. Begum *et al*. (2020)

reported that ethanolic extracts of *P. hysterophorus* roots, stems and leaves contained amino acids, fixed oils, alkaloids, flavonoids, phenols, carbohydrates, proteins and triterpenoids. Jaiswal *et al*. (2022) identified multiple phytochemicals through TLC analysis, where hexane extracts revealed terpenoids, steroids and quinones, while methanol extracts showed pronounced phenolic and alkaloid bands. Similarly, Kumar *et al*. (2013a) detected flavonoids, terpenoids and alkaloids in flower and root extracts and Pandey *et al*. (2019) reported the presence of parthenin at an Rf value of 0.6, indicating its cytotoxic potential.

In the present study, TLC provided a simple and reliable method for detecting parthenin in solvent fractionated *P. hysterophorus* extracts, particularly in the ethanol fractions. Algfri *et al.* (2022) reported multiple spots in methanol extracts with varying Rf values depending on the solvent system and Jaiswal *et al.* (2022) identified photoactive compounds using Dragendorff's and anisaldehyde-sulphuric acid reagents. Similarly, Hernández *et al.* (2011) documented parthenin elution in chloroform:ethanol (3:1) at an Rf value of approximately 0.6, consistent with the current TLC findings. Moreover, HPLC analysis of the SCFE ethanol fraction revealed a distinct parthenin peak at 5.8 min, confirming its identity (Jambamma *et al*., 2024).

The antimicrobial sensitivity test of the SCFE ethanol fraction showed strong inhibition against *E. coli*, *S. aureus* and *S. enterica*. These results align with earlier studies: Krishnaveni *et al*. (2015) found ethanol extracts of *P. hysterophorus* inhibited *E. coli* (11 mm), Deshpande *et al*. (2017) reported moderate inhibition (5.76 mm) and Nehal *et al*. (2016) observed strong antibacterial activity in supercritical mint oil. Krishnavignesh *et al*. (2013) also noted methanol extracts inhibited *S. aureus* (16 mm) and *E. coli* (15 mm), confirming broad spectrum efficacy.

5. Conclusion

The present study demonstrates that *P. hysterophorus*, though invasive, possesses notable phytochemical and pharmacological potential. Transforming this weed into a sustainable source of bioactives offers an innovative alternative to traditional eradication methods. Supercritical fluid extraction (SCFE) proved superior for isolating heat sensitive and volatile compounds under mild conditions, ensuring faster extraction and greater compound stability than conventional techniques. Phytochemical screening confirmed key metabolites such as alkaloids, flavonoids, phenols, terpenoids, tannins and saponins, all contributing to its therapeutic efficacy. TLC and HPLC analyses validated the presence of parthenin and other bioactives with characteristic Rf (0.6) and retention (5.8 min) values. The SCFE ethanol fraction exhibited strong antimicrobial activity against *E. coli*, *S. aureus* and *S. enterica*, confirming broad spectrum antibacterial potential. Eco-friendly techniques like SCFE offer a path toward valorizing invasive plants, reducing environmental impacts and advancing drug discovery. Future studies should emphasize *invivo* efficacy, toxicity evaluation and process scaleup to support industrial applications and strengthen the link between traditional medicine and modern pharmacognosy.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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