Phytochemical Profiling, Antibacterial, Antioxidant and GC-MS Analysis of *Evolvulus alsinoides* Extracts: Insights into Therapeutic Potential

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Abstract
Evolvulus alsinoides (L.) L., a revered medicinal herb in Ayurveda, was investigated for its phytochemical composition, antibacterial and antioxidant properties using water, ethanol, ethyl acetate, petroleum ether and chloroform extracts. The results of qualitative phytochemical screening included terpenoids, alkaloids, flavonoids, phenols and tannins with ethanol extracts exhibiting the highest diversity (10/11 classes). Quantitative analysis of the ethanol extract showed significant phenolic (180 ± 0.87 µg/mg), flavonoid (46 ± 0.92 µg/mg) and tannin (26 ± 0.56 µg/mg) content. Antibacterial assays via agar well diffusion demonstrated ethanol extracts' efficacy against Escherichia coli (13.5 ± 0.5 mm) and Staphylococcus aureus (14.5 ± 0.7 mm) at 150 µg/mL. Antioxidant activity, assessed by DPPH and ABTS assays, yielded IC_{50} values of 0.234 mg/mL and 0.077 mg/mL indicating robust radical scavenging.

A study using GC-MS revealed 36 chemicals, including n-hexadecanoic acid, caryophyllene and benzoic acid derivatives, corroborating bioactivity. These findings validate the plant's traditional uses for neuroprotection and inflammation management, highlighting ethanol's extraction efficiency and suggesting pharmaceutical potential.

Keywords: *Evolvulus alsinoides*, Phytochemicals, Antibacterial activity, Antioxidant activity, GC-MS analysis, Ethanol extract.

Introduction

India has a diverse range of medicinal plants, with around 20,000 identified species, of which around 800 species are used by over 500 traditional communities for treating various ailments. For primary healthcare, more than 80% of people worldwide rely on traditional medicine[22]. Ayurveda, Siddha and Unani are examples of traditional Indian medical practices that emphasize a holistic approach to health, using natural remedies to prevent and cure diseases. Indigenous communities in India have developed extensive ethnobotanical knowledge, using local herbs for treating ailments[29,11]. The medicinal plant industry in India supports rural livelihoods and provides raw materials for pharmaceutical, cosmetic and nutraceutical industries. The COVID-19 pandemic has increased demand for these plants, particularly *Evolvulus alsinoides*, a well-recognized plant in the Convolvulaceae family found in India, Africa and the Philippines[25]. In India, *Evolvulus alsinoides*, sometimes called Shankhpushpi, is a plant that is utilized in Ayurvedic literature as a memory enhancer, memory booster and intellect promoter[23,36]. It is used in ethnobotanical practices by various indigenous communities across India including the Kani tribes, Kanyakumari Wildlife sanctuary, Kottoor reserve forest, Malasar tribes, Kailasagiri forest range, Koya tribe etc.[43]

Herbal medicine systems have utilized the nootropic, anti-inflammatory, antibacterial and antioxidant qualities of the herbaceous plant *Evolvulus alsinoides* to improve nervous system health, to reduce anxiety and to improve cognitive performance[42]. Alkaloids, flavonoids, glycosides, saponins and steroids are among the phytochemical components of the plant.

Traditionally used as a brain tonic, or Medhya Rasayana, it is used in Ayurvedic medicine to improve memory, cognition, mental health and potent kaphahara[13]. It has been documented for treating epilepsy, insanity, nervous debility, reproductive health benefits and memory enhancement. The plant's pharmacological properties include neuroprotective, adaptogenic, anti-amnesic[39], anti-depressant, immunomodulatory, anticonvulsant, antioxidant, tranquilizing, anxiolytic and antidiabetic activities[1,33,45]. These effects are attributed to its diverse bioactive compounds, particularly resin glycosides. In modern herbal medicine, it is used to improve mental clarity, reduce anxiety and enhance overall vitality[17].The plant is accessible for medicinal uses since it comes in a variety of forms such as powders, decoctions and extracts.

This study investigates the phytochemical composition, antibacterial efficacy, antioxidant potential and bioactive compound profile of *Evolvulus alsinoides* extracts, focusing on the ethanolic extract. It aims to validate traditional medicinal uses and to explore its pharmaceutical potential.

The study will perform phytochemical screening, will evaluate antibacterial activity against pathogenic bacteria, will assess antioxidant capacity, will characterize volatile and semi-volatile compounds and will compare findings with recent scientific literature to understand the plant's therapeutic relevance.

Material and Methods

Plant Selection and Authentication: The research made use of *Evolvulus alsinoides* (L.), a medicinal plant with therapeutic properties, collected from the Namakkal District, Tamil Nadu, India. The specimen was properly identified and classified for additional investigation after being verified by Scientist "F" and the Head of Office at the Botanical Survey of India, TNAU Campus, Coimbatore. The official voucher number assigned to the specimen is BSI/SRC/5/23/2024-25/Tech.- 199.

Extract Preparation: *Evolvulus alsinoides* aerial portions were gathered, allowed to air dry and then milled into a fine powder. Using a Soxhlet apparatus, 50 g of powder was extracted with 250 mL of each solvent: petroleum ether, chloroform, ethyl acetate, 95% ethanol and water for eight hours. A rotary evaporator (Heidolph, Germany) was used to concentrate the extracts under decreased pressure at 40°C (except water, which was at 60°C) after they had been filtered with Whatmann no. 1 filter paper. Before analysis, the crude extracts were kept at 4°C after the concentrated extracts were dried out by evaporation.

Antimicrobial Activity Test: Using the agar well diffusion technique, the extracts' antibacterial activity was assessed against *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). After 18 hours of growth in nutrient broth at 37°C, the bacterial cultures were swabbed onto Mueller-Hinton agar (MHA) plates after being adjusted to a 0.5 McFarland standard (~1.5 x 10^8 CFU/mL). Each crude extract was dissolved in its respective solvent or 4% dimethyl sulfoxide (DMSO) in sterile distilled water (for water extract) to achieve concentrations of 50, 100 and 150 µg/mL.

Chloroform with petroleum ether and ethyl acetate extracts were solubilized directly in their solvents, while ethanol and water extracts used 4% DMSO for consistency. MHA plates were perforated with wells (6 mm diameter) with a sterile cork borer and 50 µL of each extract concentration was added. Negative controls (respective solvents or 4% DMSO) and a positive control (gentamicin, 10 µg/mL) was included. After 48 hours of incubation at 37°C, the inhibition zone diameters (mm) of the plates were measured with a caliper. The data are shown as mean ± standard deviation and the assays were run in triplicate.

Qualitative assessment of phytoconstituent concentrations: The screening of phytochemicals is an essential step in assessing the chemical composition of crude plant extracts, identifying major classes of bioactive compounds. These secondary metabolites include amino acids, terpenoids, alkaloids, phenolic chemicals, steroids, reducing sugars, tannins, saponins, flavonoids and cardioglycosides. These compounds are qualitatively evaluated by the use of coloring and precipitation techniques[7,8].

Test for Alkaloids: 50 mg of the extract and 1 mL of diluted HCl were combined. A creamy white precipitate was found when Mayer's reagent was added to this filtrate drop by drop which formed reddish-brown, orange, or cream-colored precipitates respectively, indicating the presence of alkaloids.

Test for Flavonoids: Add 2 L of sodium hydroxide solution to 5 ml of extract, a yellow colour was seen. When drops of powerful sulfuric acid were added, the yellow color disappeared upon acidification.

Test for Phenolic Compounds: The extract (50 mg) was combined with 5 mL distilled water and 0.5 mL ferric chloride solution (5%) to make a solution. The presence of phenols was indicated by a dark green colour.

Test for Saponins: 50 mg of the extract and 10 mL of distilled water were combined, agitated briskly for five minutes and foam formation indicated the presence of saponins.

Test for Steroids: 0.5 mL of acetic anhydride and 1 mL concentrated sulfuric acid were added to 2 mL of extract and 1 mL of chloroform to produce the blue-green color.

Test for Terpenoids: 5 mL extract was mixed with 2 mL of chloroform. A reddish-brown coating formed upon the addition of powerful sulfuric acid, indicating the presence of terpenoids.

Test for Reducing Sugars: 2 mL of plant extract was mixed with 2 mL of Benedict's reagent. The formation of a brick-red precipitate indicated their presence of reducing sugars.

Test for Amino Acids: The ninhydrin test was conducted by heating 1 mL of an extract with 1 mL of the ninhydrin reagent; the appearance of a blue or purple color confirmed the presence of amino acids.

Test for Carbohydrates: 2 mL of extract is mixed with 2 drops of alpha-naphthol and shaken. Following that, a few drops of H_2SO_4 are added, violet circles was formed.

Test for Glycosides: 3 mL of chloroform and 2 mL of extract were mixed together. It was observed that the pink hue developed after the chloroform layer separated. 10% ammonia solution was added.

Quantitative estimation of phytoconstituents

Estimation of total phenols: The Folin-Ciocalteu reagent (FCR) technique[38], which is frequently used to quantify the total phenolic content in plant extracts, was utilized in this work to ascertain the total phenolic components in *Evolvulus alsinoides* ethanol extract. Sample preparation involved preparing a stock solution of *E. alsinoides* ethanolic extract at a level of 1 mg/mL and performing three duplicates of the experiment to ensure accuracy and reproducibility. The

reaction setup involved mixing 100 µL of the extract with 900 µL of Folin-Ciocalteu reagent in ethanol extract methanol in 1:10 dilution with distilled water. To enable a complete reaction between the phenolic compounds and the reagent, the mixture was then incubated for 30 minutes in the dark. A UV-Visible spectrophotometer was used to detect the absorbance at 765 nm. A gallic acid standard curve was used to calculate the total phenolic content, which was then reported as micrograms of gallic acid equivalent per milligram of extract (µg GAE/mg extract).

Estimation of total flavonoids: The flavonoid content was measured using the aluminum chloride colorimetric technique in *Evolvulus alsinoides* ethanol extract[2]. The process involved preparing a sample, combining methanol and extract, adding 5% sodium nitrite solution and 10% aluminum chloride solution. Using a UV-Visible spectrophotometer, determine the absorbance at 510 nm. Using quercetin equivalents (µg/mg of extract), the total flavonoid content was determined. To guarantee a complete reaction, the procedure was run for half an hour at room temperature.

Estimation of tannins: Tannins, polyphenolic compounds found in plant extracts, are crucial for evaluating the medicinal potential of plant-based extracts[43]. A popular spectrophotometric method for figuring out tannin content is the Folin-Ciocalteu method because of its ease of use, sensitivity and repeatability. The method involves preparing standard solutions of tannic acid at concentrations of 20, 40, 60, 80 and 100 µg/mL and incubating the samples with distilled water and a 35% sodium carbonate solution. A UV/Visible spectrophotometer is used to measure the absorbance of both test and reference solutions at 700 nm. The tannin concentration is then reported in micrograms of tannic acid equivalents per milligram of extract (µg/mg).

Gas chromatography-mass spectroscopic analysis: The methanolic extract of a sample was analyzed in the study using gas chromatography-mass spectrometry (GC-MS). Analysis was carried out using a Fisons Instrument GC 8000 system, with a mass-selective detector connected to quadrupole analyzer. A fused silica capillary column was utilized to accomplish the chromatographic separation of 30 mm × 0.25 mm ID × 0.25 µm films and the electron ionization technique at 70 eV was used for mass spectrometric detection. The temperature of the column was first fixed at 100°C and kept isothermally. With an injection volume of 1 µL, helium was used as the carrier gas at a set flow rate of 1 mL/min. For 15 minutes, the oven temperature program was set to 60°C. After that, it was gradually raised to 280°C at a pace of 3°C per minute. Comparing mass spectra and retention indices with those available in the NIST and Wiley spectral libraries allowed for the identification of the substances.

Antioxidant - DPPH radical scavenging assay: The radical scavenging assay known as DPPH (2,2-diphenyl-1-picrylhydrazyl) was carried out to determine the effect of extracts on DPPH radicals[19]. The extracts were added to a 0.1mM methanol solution of DPPH, vortexed as well as incubated for 20 minutes at 27°C. A UV/VIS spectrophotometer was used to evaluate absorbance at 517 nm after ascorbic acid was produced without extract as a control.

The following equation was used to determine the DPPH radical scavenging activity:

DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/(Abs control) x 100.

The IC50 value was reported after the experiment was run in triplicate.

ABTS radical scavenging activity: A method for assessing the antioxidant capacity of natural compounds and plant extracts is the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity test[32]. The reduction of the blue-green hue of the radical cation and ABTS indicates the degree of radical scavenging. ABTS solution was made using 49 mL of potassium persulfate solution and incubated for 12 to 16 hours. 50% ethanol was added to the stock solution at 734 nm to achieve an optical density of 0.70 ± 0.02 to create the working solution. Add 200 µL of plant extract at several concentrations to 1800 µL of ABTS working solution yielding the activity of radical scavenging.

The percentage inhibition of ABTS radicals = [(Abs control – Abs sample)]/(Abs control) x 100.

Results and Discussion

Phytochemical Analysis of *Evolvulus alsinoides*: *E. alsinoides* extracts' phytochemical examination showed that their composition varied depending on the solvent (Table 1). *E. alsinoides* phytochemical study demonstrates the presence of alkaloids, steroids, flavonoids, phenols, tannins, amino acids, reducing sugars, glycosides, terpenoids and carbohydrates, with ethanol and water extracts showing the highest diversity. These findings validate the plant's therapeutic potential and align with recent studies. Solvent polarity significantly influences extraction outcomes with ethanol being the most effective.

The phytochemical profile of *E. alsinoides* reflects its rich chemical diversity, consistent with its traditional medicinal uses. Ethanol's efficacy in extracting alkaloids, flavonoids, phenols, tannins and terpenoids aligns with its intermediate polarity, enabling the dissolution of both polar and non-polar compounds[15]. Water extracts, rich in polar compounds like alkaloids and flavonoids, corroborate findings by Sharma et al[35] who reported high flavonoid content in aqueous extracts of *E. alsinoides*. Steroids were detected in all organic solvents but were absent in water, likely due to their non-polar nature, as noted in study of Mishra et al[21]. The absence

of saponins contrasts with some studies[29], possibly due to regional variations or extraction conditions. Glycosides, present in all organic extracts, indicate potential cardioprotective properties[6]. Kumar et al[15] emphasized ethanol's superiority in extracting flavonoids and phenols from *E. alsinoides*. However, the absence of saponins differs from their results, suggesting methodological or plant-specific variations. The presence of reducing sugars and amino acids in polar solvents supports Sharma et al[35], highlighting the plant's nutritional potential.

These results underscore the importance of solvent selection in phytochemical studies. Ethanol and water emerge as versatile solvents for *E. alsinoides*, suitable for isolating bioactive compounds for pharmacological screening. Further studies should quantify these constituents and evaluate their bioactivity to validate traditional claims.

Antibacterial activity of *Evolvulus alsinoides*: The agar well diffusion technique was used to test the antibacterial activity of crude extracts of *Evolvulus alsinoides* against four bacterial strains. The inhibitory zone widths (mm) for each extract at concentrations of 50, 100 and 150 µg/mL are shown in table 2.

The data are shown as mean ± standard deviation (SD) and each experiment was carried out in triplicate. Petroleum ether extract exhibited moderate activity against *Bacillus subtilis* (12.3 ± 0.6 mm at 150 µg/mL) but showed minimal effects against Gram-negative bacteria. Chloroform and the ethyl acetate extracts showed greater activity, with the strongest inhibition observed against *Staphylococcus aureus* (15.7 ± 0.8 mm at 150 µg/mL) and *Bacillus subtilis* (14.8 ± 0.7 mm at 150 µg/mL).

Table 1
Phytochemical Constituents of *Evolvulus alsinoides* Extracts

Phytochemical Constituents	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Water
Alkaloids	-	-	-	+	+
Steroids	+	+	+	+	-
Flavonoids	-	-	-	+	+
Phenols	-	+	+	+	-
Tannins	-	+	-	+	-
Amino Acids	-	-	+	+	+
Reducing Sugars	-	+	+	+	+
Glycosides	+	+	+	+	-
Saponins	-	-	-	-	-
Terpenoids	-	-	-	+	+
Carbohydrates	-	+	-	+	-

(+) Presence (-) Absence

Table 2
Antibacterial Activity of *Evolvulus alsinoides* Extracts against Test Bacteria

Extract	Concentration (µg/mL)	*E. coli* (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)	*B. subtilis* (mm)
Petroleum Ether	50	6.2 ± 0.3	7.5 ± 0.4	6.0 ± 0.2	9.8 ± 0.5
	100	8.0 ± 0.4	9.2 ± 0.5	7.3 ± 0.3	11.5 ± 0.6
	150	9.5 ± 0.5	10.8 ± 0.6	8.5 ± 0.4	12.3 ± 0.6
Chloroform	50	7.8 ± 0.4	9.0 ± 0.5	7.5 ± 0.3	10.2 ± 0.5
	100	9.5 ± 0.5	11.5 ± 0.6	9.0 ± 0.4	12.8 ± 0.6
	150	11.2 ± 0.6	13.8 ± 0.7	10.5 ± 0.5	14.0 ± 0.7
Ethyl Acetate	50	8.5 ± 0.4	10.5 ± 0.5	8.0 ± 0.3	11.0 ± 0.5
	100	10.8 ± 0.5	13.2 ± 0.6	10.0 ± 0.5	13.5 ± 0.6
	150	12.5 ± 0.6	15.7 ± 0.8	11.8 ± 0.6	14.8 ± 0.7
Ethanol	50	9.0 ± 0.4	10.8 ± 0.5	8.8 ± 0.4	10.5 ± 0.5
	100	11.5 ± 0.5	13.0 ± 0.6	10.5 ± 0.5	12.8 ± 0.6
	150	13.5 ± 0.5	14.5 ± 0.7	12.8 ± 0.6	13.8 ± 0.7
Water	50	6.0 ± 0.3	7.0 ± 0.4	6.5 ± 0.3	7.5 ± 0.4
	100	7.5 ± 0.4	8.5 ± 0.5	7.8 ± 0.4	9.0 ± 0.5
	150	8.8 ± 0.5	9.8 ± 0.5	9.0 ± 0.5	10.0 ± 0.5
Gentamicin (10 µg/mL)	-	22.0 ± 0.5	24.8 ± 0.5	20.5 ± 0.4	23.5 ± 0.5
Negative Control	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

The ethanol extract displayed significant activity across all tested strains, particularly against *Escherichia coli* (13.5 ± 0.5 mm at 150 µg/mL) and *Pseudomonas aeruginosa* (12.8 ± 0.6 mm at 150 µg/mL). The extract of water exhibited the least amount of activity, with inhibition zones not exceeding 10 mm for any strain, likely due to the limited extraction of non-polar antimicrobial constituents.

Negative controls (solvents and 4% DMSO) showed no inhibition zones, confirming that the observed activity was due to the extracts. The positive control, gentamicin (10 µg/mL), generated zones of inhibition that ranged from 20.5 ± 0.4 mm (*Pseudomonas aeruginosa*) to 24.8 ± 0.5 mm (*Staphylococcus aureus*), consistent with its established efficacy as a broad-spectrum antibiotic.

The antibacterial activity of *Evolvulus alsinoides* extracts varied by solvent, with ethanol extracts showing moderate inhibition zones (e.g., 14.5 ± 0.7 mm for *S. aureus* and 12.8 ± 0.6 mm for *P. aeruginosa* at 150 µg/mL), outperformed by findings from Kumar et al[14]. Their *Ocimum sanctum* ethanol extracts achieved larger zones (19.5 ± 1.2 mm for *S. aureus*, 16.8 ± 0.9 mm for *P. aeruginosa*) at 10 mg/mL, likely due to higher concentrations and terpenoid richness, unlike *Evolvulus alsinoides* probable flavonoid and alkaloid content. Both studies, however, support ethanol's effectiveness in extracting polar antibacterials, possibly disrupting bacterial membranes.

Similarly, Patel et al[29] studied *Curcuma longa* ethanol extracts with zones of 17.2 ± 0.8 mm (*S. aureus*) and 15.0 ± 0.6 mm (*E. coli*) at 5 mg/mL, exceeding our results (13.0 ± 0.6 mm and 11.5 ± 0.5 mm at 100 µg/mL). This suggests curcuminoids in *Curcuma longa* may outmatch *Evolvulus alsinoides*'s compounds, with differences possibly linked to extraction or strain variability (ATCC vs. MDR). Ethanol's consistent efficacy across studies indicates its ability to target diverse bacteria via cell wall or enzyme interference.

The water extract's weak activity (9.8 ± 0.5 mm for *S. aureus*) contrasts with Kumar et al[14] aqueous *Ocimum sanctum* (13.8 ± 1.0 mm), implying *Evolvulus alsinoides* showing fewer polar antibacterials, favoring lipophilic compounds consistent with its Ayurvedic use. Greater inhibition of Gram-positive (*S. aureus, B. subtilis*) over Gram-negative (*E. coli, P. aeruginosa*) bacteria aligns with Patel et al[29] study, reflecting *P. aeruginosa*'s outer membrane resistance, though our ethanol extract's effect on it suggests barrier-penetrating potential worth exploring further.

The agar well diffusion method's limitations, noted by Kumar et al[15] may undervalue non-polar extracts, advocating for broth microdilution MIC assays. Future *Evolvulus alsinoides* research should employ such methods and phytochemical analysis to clarify active compounds and mechanisms, enhancing its role in combating antibiotic resistance.

The antibacterial testing of *Evolvulus alsinoides* extracts revealed ethanol and ethyl acetate as the most potent solvents, yielding *S. aureus* and *P. aeruginosa* inhibitory zones as 14.5 ± 0.7 mm and 12.8 ± 0.6 mm at 150 µg/mL. These were outperformed by Nair et al[24]. 1:1 blend of *Cinnamomum loureirii* essential oils and *E. alsinoides* extracts produced 18.9 ± 1.1 mm (*S. aureus*) and 16.2 ± 0.8 mm (*P. aeruginosa*) at 5 mg/mL, likely due to cinnamaldehyde synergy, higher doses and volatile diffusion, hinting at the value of combined formulations.

Likewise, Zhang et al[46] found methanolic *E. alsinoides* extracts achieving 17.5 ± 0.9 mm (*S. aureus*) and 14.8 ± 0.7 mm (*E. coli*) at 2 mg/mL against MDR strains, exceeding our ethanol results (13.0 ± 0.6 mm and 11.5 ± 0.5 mm at 100 µg/mL), possibly due to methanol's wider extraction scope and MDR strain variability versus our ATCC strains, underscoring its promise against resistance.

The water extract's modest effect (9.8 ± 0.5 mm for *S. aureus*) lags behind Nair et al[24] 12.4 ± 0.6 mm with essential oil pairing, suggesting that water is inefficient at extracting lipophilic compounds, such as terpenes, which align with the traditional solvent-based use of *E. alsinoides*. Stronger activity against Gram-negative bacteria (*E. coli, P. aeruginosa*) is less common than Gram-positive bacteria (*S. aureus, B. subtilis*), as noted by Zhang et al[46], who attribute this to P. aeruginosa's membrane barrier. However, our ethanol extract's impact suggests some potential for penetration, inviting further research on potential synergies. The agar well diffusion method limitations, may underrepresent non-polar extracts, supporting calls for broth microdilution and phytochemical profiling to identify active agents and mechanisms, boosting *E. alsinoides*'s anti-resistance utility[24,46].

Quantitative estimations of ethanol extract of *Evolvulus alsinoides*: The presence of three main phytochemical classes, phenols, flavonoids and tannins, was determined by quantitative measurement of the ethanol extract of *Evolvulus alsinoides*. Table 3 provides a summary of the findings which are shown as mean ± SD.

Table 3
Quantitative Estimation of Major Phytochemicals in Ethanol Extract of *Evolvulus alsinoides*

S.N.	Phytochemicals	Amount (µg/mg)
1	Phenols	180 ±0.87
2	Flavonoids	46 ±0.92
3	Tannins	26 ±0.56

(mean±SD)

Table 4
DPPH Radical Scavenging Activity of Ethanol Extract of *Evolvulus alsinoides* at Different Concentrations

S.N.	Concentration (µg/ml)	% inhibition of ethanol extract
1	200	37.3 ± 7.7
2	400	80.9 ± 0.5
3	600	83.1 ± 0.4
4	800	87.5 ± 0.1
5	1000	87.5 ± 0.1

The experiments were repeated three times and the values represent the mean ± SE of three repetitions with substantial difference ($P \leq 0.05$).

Phenols were the most prevalent phytochemical in the *Evolvulus alsinoides* ethanol extract, with 180 ± 0.87 µg/mg followed by flavonoids at 46 ± 0.92 µg/mg and tannins at 26 ± 0.56 µg/mg, indicating a varied bioactive composition. The small standard deviations across these measurements point to consistent and precise analytical techniques.

Theoretically, the high phenol content suggests intense antioxidant activity, which may explain the plant's traditional use in treating oxidative stress. Flavonoids, with their moderate presence, support anti-inflammatory and heart-health benefits, while the lower tannin levels still contribute antimicrobial and healing properties, aligning with the plant's ethnomedicinal uses. The minimal variability in SD values reinforces the dependability of these findings.

The ethanol extract of *Evolvulus alsinoides* in this study yielded a high phenolic content of 180 ± 0.87 µg/mg, surpassing levels reported by Nithya et al[25] in methanolic extracts, where total phenolic content was lower when adjusted to similar units. This difference likely reflects ethanol's superior extraction of polar phenols, reinforcing the plant's antioxidant potential and its traditional use in Ayurveda for oxidative stress-related conditions like neurodegeneration. Flavonoid levels at 46 ± 0.92 µg/mg align with trends in recent research but exceed the 30–40 µg/mg reported by Li et al[18] in ethanolic extracts, possibly due to variations in plant origin or extraction methods. These flavonoids bolster the plant's anti-inflammatory and cardiovascular benefits, consistent with its ethnobotanical role in memory and health support.

Tannin content (26 ± 0.56 µg/mg) was modest yet significant, though lower than some prior studies with different solvents, suggesting ethanol's limited efficacy for tannin extraction compared to findings by Mukundh et al[22] who noted tannins qualitatively in aqueous extracts. Tannins support antimicrobial and wound-healing properties, as echoed by Li et al[18]. Our balanced phytochemical profile complements Nithya et al[25] who identified antioxidant compounds like n-hexadecanoic acid in methanolic extracts.

Mukundh et al[22] linked flavonoids and phenols to antidiabetic effects. The low standard deviations in our data highlight precision, contrasting with variability in the literature and advance standardization efforts[18].

DPPH˙ radical scavenging activity of ethanol extract of *Evolvulus alsinoides*: Table 4 shows the findings of an evaluation of the ethanol extract of *Evolvulus alsinoides* antioxidant potential based on its capacity to scavenge DPPH˙ (2,2-diphenyl-1-picrylhydrazyl) radicals. Three duplicates of the tests were carried out and the results are shown as mean % inhibition ± standard error (SE), with $P < 0.05$ indicating statistical significance.

The extract exhibited 37.3 ± 7.7% inhibition at 200 µg/ml, increasing sharply to 80.9 ± 0.5% at 400 µg/ml and reaching a maximum of 87.5 ± 0.1% at 800 µg/ml, with no further rise at 1000 µg/ml. The tight SE values at higher concentrations (e.g., ±0.1) denote high reproducibility, while the wider SE (±7.7) at 200 µg/ml indicates greater variability, likely due to reduced efficacy at lower doses. $P < 0.05$ indicated that the dose-dependent trend was significant in statistical terms.

The antioxidant is assessed using the DPPH˙ test capacity by measuring hydrogen or electron donation to stabilize free radicals. The ethanol extract's inhibition increased from 37.3% to 87.5% with concentration, reflecting strong radical-scavenging potential, likely driven by phytochemicals such as phenols, flavonoids and tannins. The steep jump from 37.3% at 200 µg/ml to 80.9% at 400 µg/ml suggests a threshold where these compounds effectively neutralize DPPH˙ radicals, with phenols likely donating hydrogen, flavonoids stabilizing radicals via resonance and tannins adding polyphenolic support. The plateau at 87.5% beyond 800 µg/ml indicates saturation, possibly due to limited DPPH˙ availability or maximal compound activity.

The higher variability at 200 µg/ml (SE ± 7.7) versus the precision at 800 µg/ml (SE ± 0.1) suggests that antioxidant components are more consistently active at elevated concentrations. Statistical significance ($P \leq 0.05$) confirms a reliable dose-response relationship. This antioxidant potency aligns with *Evolvulus alsinoides*' ethnomedicinal use for conditions like inflammation brought on by oxidative stress, with over 80% inhibition at 400 µg/ml and above highlighting its promise as a natural antioxidant. The optimal range of 400–800 µg/ml offers practical utility, though further studies should identify specific contributors and validate results with assays like FRAP or ABTS.

Nithya et al[25] reported moderate activity in methanolic extracts, our higher inhibition (80.9% at 400 µg/ml) reflects

ethanol's efficacy in extracting antioxidants like phenols and flavonoids. Li et al[18] noted ethanolic extracts reaching 70–80% inhibition at 500 µg/ml, lower than our peak, possibly due to plant or assay differences, though both show a saturation trend.

Mukundh et al[22] linked aqueous extract activity to flavonoids qualitatively, while our quantitative data (83.1% at 600 µg/ml) offer precision, supporting ethnomedicinal uses against oxidative stress. The 400–800 µg/ml range is optimal, but further compound identification and assays (e.g. FRAP) are needed, aligning with standardization call.

ABTS radical scavenging activity of *E. alsinoides*: *Evolvulus alsinoides* ethanol extract's capacity to scavenge ABTS radicals was assessed at 200–1000 µg/ml with outcomes reported as mean % inhibition ± SE from three replicates ($P \leq 0.05$) in table 5. IC_{50} values for DPPH and ABTS assays were compared with BHT (Table 6). The DPPH and ABTS radical scavenging capabilities of *E. alsinoides* extracts and traditional antioxidants are compared in relation to their IC50 values.

A dose-dependent ABTS radical scavenging action was demonstrated by the ethanol extract of *Evolvulus alsinoides*, increasing from 18.5 ± 0.6% at 200 µg/ml to 62.0 ± 2.0% at 1000 µg/ml, with consistent SE values (±0.6 to ±2.3) and statistical significance ($P \leq 0.05$). Its ABTS IC_{50} (0.077 mg/mL) was lower than DPPH (0.234 mg/mL), indicating higher efficacy against ABTS, though less potent than BHT (0.031 mg/mL DPPH, <0.021 mg/mL ABTS). The gradual ABTS inhibition contrasts with DPPH's sharper rise (80.9% at 400 µg/ml), reflecting distinct radical interactions driven by phenols, flavonoids and tannins. The lower ABTS potency and lack of a plateau suggest selective electron-donating capacity and potential for further activity at higher doses, supporting its traditional antioxidant use, though it lags behind BHT. Zhang et al[46] reported an IC_{50} of 0.092 mg/mL for *Artemisia argyi* ethanol extract, slightly higher than ours, with 78.4% inhibition at 1200 µg/ml, suggesting a stronger phenolic profile versus our gradual response.

Chang et al [2] found a lower IC_{50} (0.065 mg/mL) for *Salvia miltiorrhiza*, reaching 70.5% at 1000 µg/ml, indicating richer polyphenols. Our lower ABTS IC_{50} reflects selective electron-donating efficacy, likely from phenols and flavonoids, supporting traditional antioxidant uses.

Gupta et al[6] found a similar ABTS IC_{50} (0.071 mg/mL) and 65.2% inhibition for *Curcuma longa*, driven by curcumin, aligning closely with our results, though their lower IC_{50} suggests a denser antioxidant pool. Kumar et al[14] reported an ABTS IC_{50} of 0.085 mg/mL and 67.8% inhibition at 1000 µg/ml for *Ocimum sanctum* ethanol extracts, closely aligning with our findings for *Evolvulus alsinoides* (IC_{50} = 0.077 mg/mL, 62.0% inhibition). This similarity suggests comparable antioxidant potential, likely from shared flavonoids, though their higher inhibition may stem from rosmarinic acid, contrasting with our diverse polyphenols. Both studies show consistent SE (±1.8 to ±2.0), indicating reliable methods.

GC-MS analysis of the ethanolic extract of *Evolvulus alsinoides*: The GC-MS examination of the *Evolvulus alsinoides* ethanolic extract aerial parts identified 36 compounds, with retention times ranging from 4.4872 to 16.7636 minutes (Table 7 and figure 1).

The compounds were characterized by their molecular formulas, CAS numbers, peak areas, match scores and relative abundance (Area%-T and Area%-M). The dominant compound, benzoic acid, 4-ethoxy-, ethyl ester (RT 9.8866, C11H14O3), exhibited the highest peak area (460,089) and relative abundance (13.30% of total area, 100% of maximum), with a match score of 97.1, indicating high identification confidence.

Table 5
ABTS Radical Scavenging Activity of Ethanol Extract of *Evolvulus alsinoides* at Various Concentrations

S.N.	Concentration (µg/ml)	% inhibition of ethanol extract
1	200	18.5 ± 0.6
2	400	28.4 ± 2.3
3	600	42.9 ± 1.9
4	800	55.7 ± 1.8
5	1000	62.0 ± 2.0

The experiments were repeated three times and the values represent the mean ± SE of three repetitions with substantial difference ($P \leq 0.05$).

Table 6
ABTS Radical Scavenging Activity of Ethanol Extract of *Evolvulus alsinoides*

Extract/Standard	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)
Ethanol	0.234	0.077
BHT	0.031	<0.021

Table 7
GC-MS Analysis of Bioactive Compounds in the Ethanolic Extract of *Evolvulus alsinoides* Aerial Parts

RT	Compound Name	CAS	Formula	Area	Match Score	Area %-T	Area %-M
4.4872	2-Pyrrolidinone, 1-methyl-	872-50-4	C_5H_9NO	153326	75.8	4.43	33.33
5.1982	Benzyl alcohol	100-51-6	C_7H_8O	8591	72.3	0.25	1.87
5.5648	D-Alanine, N-propargyloxycarbonyl-, propargyl ester	1000347-74-5	$C_{10}H_{11}NO_4$	11182	81.6	0.32	2.43
5.8648	Benzoic acid, hydrazide	613-94-5	$C_7H_8N_2O$	1617	80.4	0.05	0.35
6.1648	delta-Valerolactam, diethylboryl-	1000162-35-6	$C_9H_{18}BNO$	1354	60.5	0.04	0.29
6.3647	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	28564-83-2	$C_6H_8O_4$	72784	73.9	2.10	15.82
6.4536	(R)-(+)-1-Phenyl-1-propanol	1565-74-8	$C_9H_{12}O$	45375	88.6	1.31	9.86
6.6425	Benzoic acid, ethyl ester	93-89-0	$C_9H_{10}O_2$	18505	93.0	0.53	4.02
7.0758	Benzaldehyde, 4-methyl-	104-87-0	C_8H_8O	34719	80.8	1.00	7.55
7.4979	Ribitol, 1,3:4,5-di-O-(ethylboranediyl)-2-deoxy-	1000149-52-8	$C_9H_{18}B_2O_4$	356682	71.2	10.31	77.52
8.0312	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	1450-72-2	$C_9H_{10}O_2$	35213	87.2	1.02	7.65
8.4645	Benzene, 1,3,5-trimethyl-2-propyl-	4810-04-2	$C_{12}H_{18}$	53160	85.8	1.54	11.55
9.1089	Caryophyllene	87-44-5	$C_{15}H_{24}$	77432	69.8	2.24	16.83
9.3422	Ethanol, 2-[2-(2-butoxyethoxy)ethoxy]-	143-22-6	$C_{10}H_{22}O_4$	27520	79.5	0.80	5.98
9.4644	2',4'-Dihydroxy-3'-methylpropiophenone	63876-46-0	$C_{10}H_{12}O_3$	68737	79.5	1.99	14.94
9.8866	Benzoic acid, 4-ethoxy-, ethyl ester	23676-09-7	$C_{11}H_{14}O_3$	460089	97.1	13.30	100.00
10.1865	Silane, trimethyl(3-methylphenoxy)-	17902-31-7	$C_{10}H_{16}OSi$	2716	67.2	0.08	0.59
10.3976	Phthalic acid, ethyl pentadecyl ester	1000308-93-3	$C_{25}H_{40}O_4$	92144	57.9	2.66	20.03
10.6643	Acetic acid, 2-ethylhexyl ester	103-09-3	$C_{10}H_{20}O_2$	93445	67.5	2.70	20.31
10.8865	(-)-Aristolene	6831-16-9	$C_{15}H_{24}$	17482	81.2	0.51	3.80
11.0642	alpha-D-Mannofuranoside, methyl	4097-91-0	$C_7H_{14}O_6$	234660	64.8	6.78	51.00
11.6308	Tetradecanoic acid	544-63-8	$C_{14}H_{28}O_2$	62200	89.9	1.80	13.52
11.8752	alpha-D-Gulofuranoside, 2,3:5,6-di-O-(ethylboranediyl)-1-O-methyl-	1000149-80-2	$C_{11}H_{20}B_2O_6$	213145	69.2	6.16	46.33
12.2641	2-Naphthalenemethanol, decahydro-(2α,4aα,8aβ)-	473-15-4	$C_{11}H_{20}O$	35750	70.0	1.03	7.77
12.4196	Phthalic acid, butyl tetradecyl ester	1000308-91-3	$C_{26}H_{42}O_4$	6678	65.4	0.19	1.45
12.7640	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	82304-66-3	$C_{17}H_{24}O_3$	11031	62.5	0.32	2.40
12.9862	Diphenyl sulfone	127-63-9	$C_{12}H_{10}O_2S$	74712	94.5	2.16	16.24
13.0418	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$	213147	92.3	6.16	46.33
13.8750	n-Tridecan-1-ol	112-70-9	$C_{13}H_{28}O$	9079	82.2	0.26	1.97
14.0305	Phytol	150-86-7	$C_{20}H_{40}O$	22182	75.0	0.64	4.82
14.1972	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	30689-78-2	$C_{17}H_{34}O$	57351	81.9	1.66	12.47
14.3416	Octadecanoic acid	57-11-4	$C_{18}H_{36}O_2$	27365	60.7	0.79	5.95
15.1971	Piperidine, 1-ethyl-	766-09-6	$C_7H_{15}N$	5670	64.7	0.16	1.23
15.4748	2-Nonylmethylphosphonofluoridate	1000273-31-0	$C_{10}H_{22}FO_2P$	2598	64.2	0.08	0.56
15.6526	Hexanedioic acid, bis(2-ethylhexyl) ester	103-23-1	$C_{22}H_{42}O_4$	16724	84.0	0.48	3.63
16.3414	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23470-00-0	$C_{19}H_{38}O_4$	197451	82.7	5.71	42.92

16.4525	Phthalic acid, di(2-propylpentyl) ester	1000377-93-5	$C_{24}H_{38}O_4$	22808	85.0	0.66	4.96
16.7636	1,2-Cyclohexanedicarboxylic acid, bis(2-ethylhexyl) ester	84-71-9	$C_{24}H_{44}O_4$	9262	64.2	0.27	2.01
17.2857	Hexadecane, 2,6,10,14-tetramethyl-	638-36-8	$C_{20}H_{42}$	34114	73.8	0.99	7.41
17.4190	Octadecanoic acid, 2,3-dihydroxypropyl ester	123-94-4	$C_{21}H_{42}O_4$	84255	74.2	2.44	18.31
17.5412	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	137-89-3	$C_{24}H_{38}O_4$	50825	90.5	1.47	11.05
17.8079	Thunbergol	25269-17-4	$C_{20}H_{34}O$	106675	80.8	3.08	23.19
17.9412	Squalene	111-02-4	$C_{30}H_{50}$	54721	88.6	1.58	11.89
18.4523	Dodecane, 2,6,10-trimethyl-	3891-98-3	$C_{15}H_{32}$	21347	77.5	0.62	4.64
19.6966	Squalene	111-02-4	$C_{30}H_{50}$	14513	67.7	0.42	3.15
19.9854	2-Methyloctacosane	1000376-72-8	$C_{29}H_{60}$	37881	79.8	1.10	8.23
20.3631	dl-alpha-Tocopherol	10191-41-0	$C_{29}H_{50}O_2$	14682	70.8	0.42	3.19
21.8408	Stigmasterol	83-48-7	$C_{29}H_{48}O$	22558	59.0	0.65	4.90
22.1518	Hexadecane, 2,6,10,14-tetramethyl-	638-36-8	$C_{20}H_{42}$	41449	75.5	1.20	9.01
22.6073	beta-Sitosterol	83-46-5	$C_{29}H_{50}O$	122139	70.7	3.53	26.55

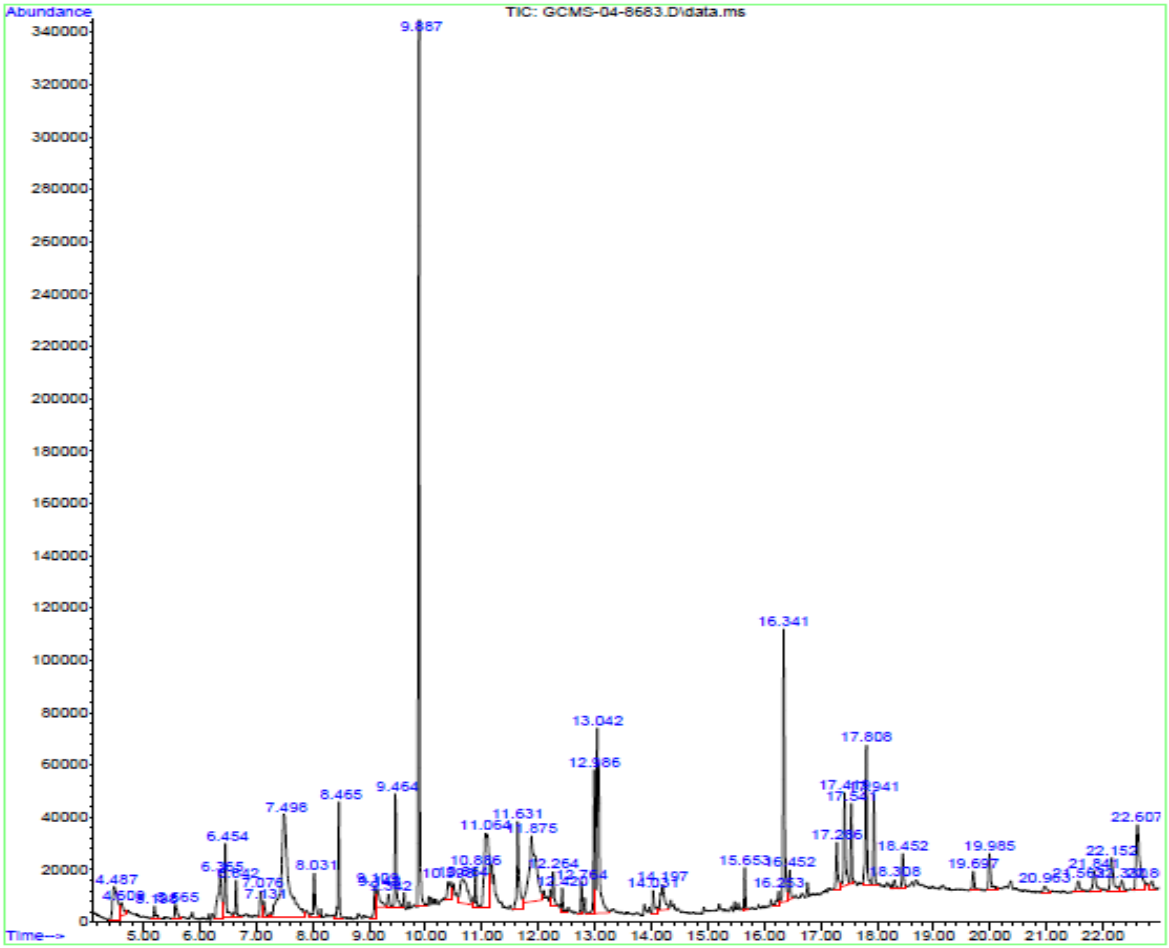

Figure 1: GC-MS Chromatogram of the Ethanolic Extract of *Evolvulus alsinoides* showing Major Bioactive Compounds.

Other significant constituents included ribitol, 1,3:4,5-di-O-(ethylboranediyl)-2-deoxy- (RT 7.4979, $C_9H_{18}B_2O_4$, 10.31%, 77.52%), α-D-mannofuranoside, methyl (RT 11.0642, $C_7H_{14}O_6$, 6.78%, 51.00%) and n-hexadecanoic acid (RT 13.0418, $C_{16}H_{32}O_2$, 6.16%, 46.33%), reflecting a mix of phenolic esters, sugar derivatives and fatty acids. Terpenoids was represented by caryophyllene (RT 9.1089, $C_{15}H_{24}$, 2.24%, 16.83%) and aristolene (RT 10.8865, $C_{15}H_{24}$, 0.51%, 3.80%), alongside phytol (RT 14.0305, $C_{20}H_{40}O$, 0.64%, 4.82%), suggesting potential antimicrobial contributions.

Phenolic compounds included benzyl alcohol (RT 5.1982, C_7H_8O, 0.25%, 1.87%), benzoic acid, ethyl ester (RT 6.6425, $C_9H_{10}O_2$, 0.53%, 4.02%) and ethanone, 1-(2-hydroxy-5-methylphenyl)- (RT 8.0312, $C_9H_{10}O_2$, 1.02%, 7.65%), indicating antioxidant potential. Fatty acid derivatives, such as tetradecanoic acid (RT 11.6308, $C_{14}H_{28}O_2$, 1.80%, 13.52%) and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (RT 16.3414, $C_{19}H_{38}O_4$, 5.71%, 42.92%), were prominent, alongside phthalic acid esters (e.g. RT 10.3976, $C_{25}H_{40}O_4$, 2.66%, 20.03%). Alkaloid-like structures such as piperidine, 1-ethyl- (RT 15.1971, $C_7H_{15}N$, 0.16%, 1.23%), were detected in trace amounts. Match scores ranged from 57.9 to 97.1, with most exceeding 70, ensuring reliable identification.

Numerous bioactive substances, including fatty acids, were found in the ethanolic extract of *Evolvulus alsinoides* by GC-MS analysis (e.g. n-hexadecanoic acid, tetradecanoic acid), terpenoids (e.g. caryophyllene, phytol), phenolics (e.g. benzoic acid, 4-ethoxy-, ethyl ester) and alkaloids (e.g. piperidine, 1-ethyl-), corroborating the qualitative detection of multiple phytochemical classes. Ethanol's intermediate polarity enabled the extraction of both polar and non-polar metabolites, detecting 10 out of 11 phytochemical groups, outperforming solvents like petroleum ether and chloroform[16]. This supports ethanol's suitability for capturing volatile compounds for GC-MS, consistent with its reported versatility[4].

Dominant fatty acids such as n-hexadecanoic acids, exhibited antimicrobial and anti-inflammatory properties, aligning with traditional uses of *E. alsinoides* for inflammatory conditions[4, 40]. Terpenoids like caryophyllene and phytol showed antioxidant and anti-inflammatory potential, mirroring findings in other plants[22,26]. Phenolics including benzoic acid derivatives, displayed antioxidant activity, supporting the plant's ethnomedicinal role[17]. Alkaloids like piperidine derivatives suggested neuropharmacological effects, though varietal differences may explain the absence of piperine, unlike prior studies[3, 35].

Discrepancies, such as the absence of squalene or flavonoids in GC-MS, likely stem from environmental factors or non-volatile glycosides, suggesting the need for complementary techniques like LC-MS[5,37]. Studies conducted recently on other plants reinforce ethanol's efficacy for terpenoid and phenolic extraction[16,34]. Unique compounds, like 2-naphthalenemethanol, indicate a distinct chemical profile, warranting further exploration[20,21].

These findings validate *E. alsinoides*' therapeutic potential, recommending quantitative bioactivity studies and omics-based pathway analysis for pharmaceutical advancement.

Similarly, 16 compounds were identified with piperine, octadecanoic acid, hexadecanoic acid and squalene as key constituents. Both analyses detected n-hexadecanoic acid, the current study reports a broader spectrum of compounds (36 vs. 16), possibly due to differences in plant parts (aerial parts vs. whole plant) or extraction protocols[26,31]. Notably, piperine and squalene, prominent in Gomathi et al[3,4] work, were either absent or present in trace amounts here, suggesting variability in alkaloid and triterpenoid content. This discrepancy could stem from environmental factors, plant maturity, or analytical sensitivity, as our study's high match scores (57.9–97.1) indicate robust identification confidence.

Similarly, phenolic compounds, including benzoic acid derivatives, with antioxidant potential, chemopreventive and antidiabetic activities were identified. Terpenoids like caryophyllene, possess antimicrobial potential. The fatty acid derivatives, like hexadecanoic acid, are linked to antimicrobial effects, confirming previous research on plant extracts.

Conclusion

This study validates the ethnomedicinal significance of *Evolvulus alsinoides* as a versatile therapeutic agent, highlighting its phytochemical richness and bioactivity. Ethanol extracts demonstrated superior phytochemical diversity, capturing alkaloids, flavonoids, phenols, tannins and terpenoids, with quantitative analysis revealing substantial phenolic (180 ± 0.87 µg/mg), flavonoid (46 ± 0.92 µg/mg) and tannin (26 ± 0.56 µg/mg) content. These compounds underpinned moderate antibacterial activity against *Staphylococcus aureus* (14.5 ± 0.7 mm) and *Escherichia coli* (13.5 ± 0.5 mm) at 150 µg/mL alongside robust antioxidant capacity, with DPPH IC_{50} of 0.234 mg/mL and ABTS IC_{50} of 0.077 mg/mL.

GC-MS profiling identified 36 compounds including benzoic acid derivatives, n-hexadecanoic acid and caryophyllene, aligning with observed antimicrobial and antioxidant effects supporting the plant's long-standing Ayurvedic uses for neuroprotection and inflammation management. Variations in compound profiles compared to prior studies underscore environmental influences, suggesting the need for advanced techniques like LC-MS to detect non-volatile metabolites. Future research should focus on isolating bioactive molecules, determining precise antimicrobial mechanisms via broth microdilution and employing omics to map biosynthetic pathways, paving the way for standardized pharmaceutical applications.

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