

Comparative Phytochemical Profiling and In-Silico Analysis of Bioactive Metabolite from *Pergularia daemia*

Rekha Anantharaman

Saveetha Medical College and Hospital (SMCH), Saveetha Institute of Medical and Technical Sciences

Alagendran Subbarayalu

Dhanalakshmi Srinivasan Agriculture College Perambalur

Harani Rajendran

³ADRC Lifesciences Pvt. Ltd & Ashwanth Diagnostics and Research Centre

Ramachandran Kavitha

Holy Cross College (Autonomous)

Sahaya Sathish

⁵PG Research Department of Botany St. Joseph College (Autonomous)

Bharathiraja Chinnapandi

adrc1820@gmail.com

Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University

Arjun Pandian

Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University

Suguna Kasirajan

Saveetha Medical College and Hospital (SMCH), Saveetha Institute of Medical and Technical Sciences, (SIMATS)

Sakthivel Muthu

Saveetha Medical College and Hospital (SMCH), Saveetha Institute of Medical and Technical Sciences, (SIMATS)

Chellasamy Panneerselvam

University of Tabuk

Abdulrahman Alasmari

University of Tabuk

Article

Keywords: Flavonoids, GC-MS, *Pergularia daemia*, Phytochemical profiling, Triterpenoids

Posted Date: October 14th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7605292/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Comparative Phytochemical Profiling and In-Silico Analysis of Bioactive Metabolite from *Pergularia daemia*

Rekha Anantharaman¹, Alagendran Subbarayalu², Harani Rajendran³, Ramachandran Kavitha⁴, Sahaya Sathish⁵, Bharathiraja Chinnapandi^{6,*}, Arjun Pandian⁶, Suguna Kasirajan⁷, Sakthivel Muthu^{8,*}, Chellasamy Panneerselvam^{9,10}, Abdulrahman Alasmari^{9,10}

¹ Centre for Global Health Research (CGHR), Department of Obstetrics and Gynaecology, Saveetha Medical College and Hospital (SMCH), Saveetha Institute of Medical and Technical Sciences, Chennai 602105, Tamil Nadu, India; microrrekha@gmail.com (R.A).

² Crop Management Division, Dhanalakshmi Srinivasan Agriculture College Perambalur -621212, Tamil Nadu, India; proteinblg@gmail.com (S.A).

³ ADRC Lifesciences Pvt. Ltd & Ashwanth Diagnostics and Research Centre, Madurai-625 018, Tamil Nadu, India; harani.r99@gmail.com (H.R).

⁴ PG & Research department of Botany, Holy Cross College (Autonomous), Tiruchirappalli- 620 002, Tamil Nadu, India; kavitharamachandran@hcctrichy.ac.in (R.K).

⁵ PG Research Department of Botany, St. Joseph College (Autonomous), Tiruchirappalli - 620002, Tamil Nadu, India; sahayasathish@yahoo.in (S.S).

⁶ Centre for Applied Research, Institute of Biotechnology, School of Science and Engineering (SSE), Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-602 105, Tamil Nadu, India; adrc1820@gmail.com (B.C) & arjungri@gmail.com (A.P).

⁷ Saveetha Medical College & Hospital (SMCH), Saveetha Institute of Medical and Technical Sciences, (SIMATS), Saveetha University, Chennai-602 105, Tamil Nadu, India; Suguna6299@gmail.com (S.K).

⁸ Department of Dermatology, Saveetha Medical College and Hospital (SMCH), Saveetha Institute of Medical and Technical Sciences, (SIMATS), Thandalam, Chennai-602 105, Tamil Nadu, India; saktthivel@gmail.com (S.M).

⁹ Department of Biology, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia; cpselva@ymail.com (C.P); ab.alasmari@ut.edu.sa (A.A)

¹⁰ Biodiversity Genomics Unit, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia.

* Corresponding Authors: adrc1820@gmail.com (B.C) & saktthivel@gmail.com (S.M)

Abstract

Pergularia daemia, commonly known as the Hair Knot Plant, is a perennial twining herb traditionally used for treating liver disorders and inflammatory conditions. This study investigated the phytochemical composition of different plant parts leaves, stems, flowers, and fruits through qualitative and quantitative screening, GC-MS analysis, and molecular docking. Phytochemical assays revealed the presence of flavonoids, tannins, alkaloids, glycosides, saponins, and terpenoids, with high levels of flavonoids (up to 48.33%) and tannins (98 µg/mL) in leaves and flowers. GC-MS analysis identified numerous bioactive compounds, including β -sitosterol, stigmasterol, α -amyrin, lupeol, palmitic acid, linolenic acid, proceroside, and loliolide compounds associated with antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial activities. Among these, α -amyrin, a major triterpenoid with strong anti-inflammatory potential, was subjected to in silico docking against TGF- β (PDB ID: 1TGK). The docking results showed a binding affinity of -6.9 kcal/mol, indicating stable interaction primarily through hydrophobic forces. This molecular interaction supports the potential role of α -amyrin in modulating inflammatory pathways. Overall, the findings validate the traditional use of *P. daemia* and highlight its promise as a source of phytotherapeutics, particularly for liver and inflammatory disorders.

Keywords: Flavonoids; GC-MS; *Pergularia daemia*; Phytochemical profiling; Triterpenoids

1. Introduction

Pergularia daemia (Forssk.) Chiov., commonly referred to as Veliparuthi, is a perennial twining herbaceous climber from the Apocynaceae family [1]. It is widely distributed across tropical and subtropical regions, particularly in countries such as India, Pakistan, and parts of Africa [2]. This plant has garnered substantial attention in ethnomedicine due to its extensive range of therapeutic applications. In traditional South Asian medicine, various parts of *Pergularia daemia* including its leaves, stems, roots, and latex have long been used to treat numerous ailments. The plant's medicinal properties are attributed to its rich phytochemical composition, which has been the focus of scientific exploration for potential pharmacological applications. The phytochemical components of *Pergularia daemia* are diverse and abundant, contributing to the plant's medicinal versatility. Key bioactive compounds include flavonoids such as kaempferol and quercetin, [3] which are particularly known for their antioxidant and anti-inflammatory properties, protecting cells from oxidative stress and modulating inflammatory responses. Alkaloids, including pergularine [4], are recognized for their analgesic effects, providing relief from pain. Steroids like β -sitosterol and stigmasterol [5] contribute significantly to the plant's anti-inflammatory potential, making it useful in conditions that involve swelling and inflammation. Additionally, tannins, saponins, terpenoids, and phenolic compounds offer unique pharmacological benefits. Together, these phytochemicals form the foundation for the plant's widespread use in traditional medicine across various parts of India, with each part used for distinct therapeutic purposes.

Each organ of *Pergularia daemia* exhibits distinct medicinal properties, which strongly suggest a compartmentalized and organ-specific distribution of bioactive phytochemicals within the plant. This distribution pattern implies that the therapeutic

potential of the plant cannot be attributed to a single constituent or plant part, but rather to a synergistic network of compounds uniquely present in specific tissues. The leaves of *P. daemia* are among the most used parts in traditional medicine. They are primarily employed for their antipyretic (fever-reducing), antidiabetic, analgesic, and anti-inflammatory effects [7]. In traditional healing systems such as Ayurveda and Siddha, leaf extracts are often applied topically to treat dermatological conditions including eczema, skin ulcers, and boils [8]. Internally, they are used to manage metabolic disorders such as diabetes mellitus, where they help regulate blood glucose levels. The presence of flavonoids and phenolic compounds in the leaves contributes significantly to their antioxidant properties, which help mitigate oxidative stress—a known contributor to chronic inflammation and metabolic dysfunction [9]. The stems have long been used in folk medicine for their laxative and anthelmintic properties [10]. Decoctions or powdered formulations of the stems are administered orally to treat digestive disorders, including constipation and intestinal worm infestations. Their purgative action is often mild and is complemented by bioactive compounds such as saponins and tannins, which may aid in gastrointestinal cleansing and antiparasitic activity. In rural communities, the stems are sometimes included in polyherbal formulations for gastrointestinal support, suggesting their relevance in traditional gastrointestinal therapy [11].

The roots of *P. daemia* are particularly renowned for their application in managing respiratory disorders, including asthma, bronchitis, and chronic cough [12]. They are typically used in the form of root decoctions or powders to relieve respiratory congestion and promote expectoration. Additionally, the roots possess diuretic properties, facilitating the elimination of excess fluids from the body, and emetic properties at higher doses, which have traditionally been used to induce

vomiting in cases of poisoning or severe indigestion [13]. Phytoconstituents such as alkaloids, glycosides, and certain terpenoids in the roots are believed to contribute to these therapeutic effects, although detailed pharmacological validation is still needed. The latex, a sticky, milky exudate secreted from the stems and leaves when the plant is cut, is another pharmacologically significant component of *P. daemia* [14]. It has been traditionally applied topically for wound healing, owing to its presumed antimicrobial, anti-inflammatory, and coagulant effects. In some traditional practices, the latex is also administered in controlled doses for the treatment of gastric ulcers, where it may act by forming a protective barrier over ulcerated mucosa or by modulating gastric secretions [15]. The exact biochemical composition of the latex is complex, potentially involving proteins, alkaloids, and latex-specific enzymes. The seeds of *P. daemia* also hold significant medicinal value. They are especially known for their anti-inflammatory action and are used in the traditional treatment of arthritis, joint pain, and rheumatism [16]. Crushed or powdered seeds are often incorporated into herbal remedies aimed at reducing inflammation and pain in musculoskeletal conditions. Although not extensively studied, the seeds are presumed to contain bioactive compounds such as steroids, fatty acids, and alkaloids, which may contribute to their therapeutic effects [17].

GC-MS has emerged as a critical analytical tool in modern phytochemistry, particularly for the identification and quantification of volatile and semi-volatile compounds in medicinal plant extracts [18]. By coupling the separation capacity of gas chromatography with the detection accuracy of mass spectrometry, GC-MS enables precise characterization of complex mixtures of secondary metabolites [19]. In the case of *Pergularia daemia*, this technique is instrumental in identifying the key phytoconstituents responsible for the plant's wide range of pharmacological activities.

Compounds such as terpenoids, flavonoids, alkaloids, and steroids which are often volatile or semi-volatile in nature can be efficiently detected and profiled using this method [20]. Such comprehensive profiling not only aids in the scientific validation of traditional knowledge but also supports future drug development initiatives. The significance of GC-MS analysis extends beyond mere identification. Quantification of bioactive compounds is crucial for evaluating the potency of plant extracts and for establishing standardized dosages in therapeutic formulations. Moreover, GC-MS plays a vital role in ensuring the quality and safety of herbal preparations by identifying the presence of contaminants, adulterants, or residual solvents [21]. In modern pharmacognosy, the technique is also used in metabolomics to generate chemical fingerprints of plant species, facilitating the standardization and authentication of plant materials [22]. These capabilities make GC-MS a preferred method in both academic and industrial settings for the analysis of medicinal plants. The present study is designed to systematically investigate the phytochemical composition of different parts of *Pergularia daemia* specifically the leaves, stems, fruits, and flowers. The methodology involves the collection of plant materials, followed by shade drying, pulverization, and ethanol-based Soxhlet extraction to obtain phytochemically rich extracts. Subsequent analysis using GC-MS aims to identify and quantify the secondary metabolites present in each organ. The core objective is to generate detailed phytochemical profiles for each extract and to compare the metabolite diversity across the various plant parts. It also aims to lay a foundation for further pharmacological studies and the development of standardized herbal formulations.

2. Methodology

2.1. Plant Collection

The plant *Pergularia daemia* was collected from T. Mettupatti, a foothill village situated in the Sirumalai hills of Tamil Nadu, India (10.1942° N, 77.9967° E). With the instruction of Plant Taxonomist **Dr. R. Ramasubbu**, Associate Professor, Department of Biology, Gandhigram Rural University, Dindigul, Tamil Nadu, India. Plant collected very limited and collected plant identified authenticated by Prof. R.R. herbarium voucher specimen number GDU1364 included. This area was specifically chosen for its diverse ecological conditions and rich biodiversity, which are known to influence the secondary metabolite composition of medicinal plants. The plant parts collected included healthy and disease-free stems, leaves, flowers and fruits. Care was taken to avoid mechanical damage during collection, and the samples were stored in clean, labeled containers to prevent cross-contamination.

2.2. Preparation of Plant Extracts

Following collection, each plant part was individually sorted and subjected to shade drying under well-ventilated conditions for several days to prevent degradation of heat- or light-sensitive phytochemicals. Once completely dried, the materials were ground into a fine powder using a mechanical grinder. The powdered samples were then stored in airtight containers until further use. Ethanolic extraction was performed using the Soxhlet apparatus, with 95% ethanol as the solvent. Separate Soxhlet extractions were carried out for the powdered stems, leaves, flowers and fruits sample [23]. The extraction process continued until the solvent in the siphon tube ran clear, indicating the exhaustion of extractable compounds. The ethanolic extracts were concentrated using a rotary vacuum evaporator and subsequently preserved at 4°C for upcoming phytochemical evaluations and GC-MS profiling.

2.3. Phytochemical Analysis

2.3.1. Qualitative Estimation [24-26]

Test for Phenols:

An equal volume (2 mL) of 5% ferric chloride solution was mixed with 2 mL of the plant extract. The development of an intense bluish-green or dark green hue confirmed the existence of phenolic constituents.

Test for Flavonoids:

Approximately 2 mL of the plant extract was treated with 0.5 mL of concentrated hydrochloric acid (36%) and a small strip of magnesium metal (around 10 mg). The emergence of pink, reddish, or orange coloration served as a positive indication of flavonoid compounds.

Test for Steroids:

Two milliliters of the extract were combined with an equal amount of chloroform (CHCl_3). Subsequently, 2 mL of concentrated sulfuric acid (98%) was carefully introduced along the inner wall of the test tube. The formation of a reddish-brown ring at the junction between the two layers signified the steroidal constituents.

Test for Glycosides:

A volume of 2 mL of the plant extract was combined with 1 mL of glacial acetic acid containing a minute quantity of ferric chloride (0.1%). Following this, 1 mL of concentrated sulfuric acid was gently added along the inner surface of the test tube. The appearance of a distinct reddish-brown or greenish-blue band at the boundary of the two liquids suggested the existence of glycosidic compounds.

Test for Tannins:

To 2 mL of the sample extract, an equal volume of a freshly made 5% iron(III)

chloride solution was added. The emergence of a dark bluish-black, olive green, or muddy green precipitate was interpreted as a positive reaction for tannins.

Test for Alkaloids:

A few drops (around 0.5 mL) of Dragendorff's reagent were added to 2 mL of the test extract. This reagent, consisting of potassium iodide and bismuth salts, reacted with alkaloid constituents to produce an orange to reddish-brown precipitate, which served as confirmation of their presence.

Test for Saponins:

Equal volumes (5 mL each) of the extract and distilled water were poured into a test tube. The mixture was shaken vigorously for two minutes. The appearance of a stable foam layer, at least 1 cm in height and lasting over ten minutes, was indicative of saponins in the sample.

Test for Carbohydrates:

To 2 mL of the plant extract, two drops of Molisch's reagent (a 10% alcoholic solution of α -naphthol) were added. Then, 1 mL of concentrated sulfuric acid was carefully added along the sides of the test tube without mixing. The formation of a violet or purple ring at the interface of the two liquids indicated the presence of carbohydrate

Test for Proteins:

A volume of 2 mL of the extract was treated with 2 mL of Biuret reagent, which comprises a combination of diluted copper sulfate and sodium hydroxide. Upon gentle mixing, the appearance of a violet hue confirmed the existence of proteins through interaction with peptide linkages

Test for Terpenoids:

To 2 mL of the extract, 2 mL of chloroform was introduced and thoroughly mixed.

Concentrated sulfuric acid (2 mL) was then cautiously added along the side of the test tube. A reddish-brown coloration emerging at the contact point of the two phases indicated the presence of terpenoid compounds.

2.3.2. Quantitative estimations

Total Flavonoid Content

In this procedure, 1 mL of the extract was combined with 3 mL of methanol. Subsequently, 0.2 mL of a 10% aluminum chloride solution, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water were added sequentially. The resulting mixture was allowed to stand at ambient temperature for 30 minutes to facilitate color development. The absorbance was then recorded at 510 nm using a UV-Visible spectrophotometer. Flavonoid levels were determined by referencing a standard curve constructed with varying concentrations of rutin, and the outcomes were expressed as milligrams of rutin equivalent per gram of extract (mg RE/g) [27].

Total Tannin Content

To estimate the tannin levels, 0.1 mL of the plant extract was first diluted with 5 mL of distilled water. Then, 0.5 mL of Folin–Ciocalteu reagent was added, followed by the introduction of 1 mL of 35% sodium carbonate solution. The volume of the mixture was made up to 10 mL using distilled water. The solution was allowed to react at room temperature for 30 minutes. After incubation, absorbance was measured at 700 nm using a UV-Vis spectrophotometer. Tannin concentration was determined by comparing the absorbance values against a standard curve constructed using tannic acid, and results were expressed as milligrams of tannic acid equivalent per gram of extract [28].

2.4. GC-MS Analysis

GC-MS analysis was carried out to profile the phytochemical constituents present in the ethanolic extracts of *Pergularia daemia* stems, leaves, flowers and fruits. Prior to the analysis, the samples were derivatized using trimethylsilyl chloride (TMS) to improve the volatility and thermal stability of the analytes, facilitating efficient separation and detection of the compounds. The derivatized extracts were analyzed using an Agilent 7890A gas chromatograph coupled to a 5975C mass selective detector. Compound separation was performed using an HP-5MS capillary column (30 meters in length, 0.25 mm internal diameter, and 0.25 μm film thicknesses). Ultra-high-purity helium served as the carrier gas, maintained at a steady flow rate of 1.0 mL per minute. A 1 μL aliquot of each derivatized extract was introduced in splitless mode. The injector temperature was set to 250°C, while the interface to the mass spectrometer was kept at 280°C. The column oven temperature was programmed to start at 60°C, then ramped at 10°C per minute until reaching 280°C, which was held constant for 10 minutes to allow for the elution of high-boiling compounds. The mass spectrometer operated in electron ionization (EI) mode at ionization energy of 70 electron volts (eV), scanning ions in the range of 50 to 600 mass-to-charge ratio (m/z). Spectral data were analyzed by matching fragmentation patterns against entries in the NIST and Wiley mass spectral libraries [29].

2.5. *In Silico* Docking Analysis of α -Amyrin

2.5.1. Preparation of Target Protein Structure

The three-dimensional structure of Human Transforming Growth Factor (PDB ID: 1TGK) was sourced from the Protein Data Bank. To prepare the protein for docking, it was pre-processed using AutoDock Tools by removing crystallographic water

molecules, incorporating polar hydrogen atoms, and applying Kollman partial charges. The finalized structure was exported in PDBQT format for docking studies.

2.5.2. Ligand Acquisition and Optimization

The molecular structure of α -Amyrin was retrieved from the PubChem database in SDF format [30]. The file was converted to PDB format using Open Babel, followed by further processing in AutoDock Tools. Gasteiger charges were assigned, rotatable bonds were defined, and the prepared ligand structure was saved in PDBQT format suitable for docking.

2.5.3. Molecular Docking Procedure

Docking simulations were performed using AutoDock Vina, which predicts ligand binding conformations through an iterative scoring and optimization algorithm. Several binding poses were generated and ranked according to their predicted binding energies (expressed in kcal/mol). The conformation with the most negative binding energy was selected for further structural evaluation.

2.5.4. Binding Interaction and Visualization

The best binding conformation of α -Amyrin with the protein target was visualized in three dimensions using PyMOL, while Discovery Studio Visualizer was employed to generate a two-dimensional interaction map. This analysis helped identify key amino acid residues involved in hydrophobic interactions, providing a molecular-level understanding of the ligand's binding mechanism [31].

3. Results

3.1. Phytochemical Analysis

3.1.1. Qualitative Estimation

The phytochemical screening of the ethanolic extracts from different parts of *Pergularia daemia* (leaf, stem, flower, and fruit) revealed a diverse distribution of

secondary metabolites (Table 1). Among the analyzed phytochemicals, flavonoids were universally present across all four parts (S1–S4), indicating their widespread role in the plant's biological activity. Tannins were observed in the leaf, stem, and flower extracts but were absent in the fruit. Glycosides were detected in the stem and flower extracts, whereas alkaloids were only present in the stem. Saponins were found in the leaf and stem, suggesting their localization in the aerial vegetative organs. Terpenoids were exclusively identified in the flower extract. Notably, phenols, steroids, carbohydrates, and proteins were not detected in any of the plant parts analyzed.

Table 1: Qualitative Phytochemical Screening of *Pergularia daemia*

Phytochemicals	Result			
	Leaf	Stem	Flower	Fruit
Phenol	-	-	-	-
Flavonoids	+	+	+	+
Steroids	-	-	-	-
Glycosides	-	+	+	-
Tannin	+	+	+	-
Alkaloids	-	+	-	-
Saponin	+	+	-	-
Carbohydrate	-	-	-	-
Protein	-	-	-	-
Terpenoids	-	-	+	-

3.1.2. Quantitative estimations

Total Flavonoid Content

The flavonoid content exhibited organ-specific distribution, the highest concentration was recorded in the flower extract (59.5 µg/mL), followed by the leaf (48.0 µg/mL), stem (35.0 µg/mL), and fruit (12.0 µg/mL). The elevated flavonoid levels in the flower and leaf extracts reflect their likely involvement in oxidative stress mitigation and potential protective functions, which further support their ethnopharmacological relevance, the standard reference rutin was, used (Fig. 1).

Table 2. Total Flavonoid Content in Different Parts of *Pergularia daemia*

Sample	Total Tannin Concentration (µg/ml)
Leaf	48
Stem	35
Flower	59.5
Fruit	12

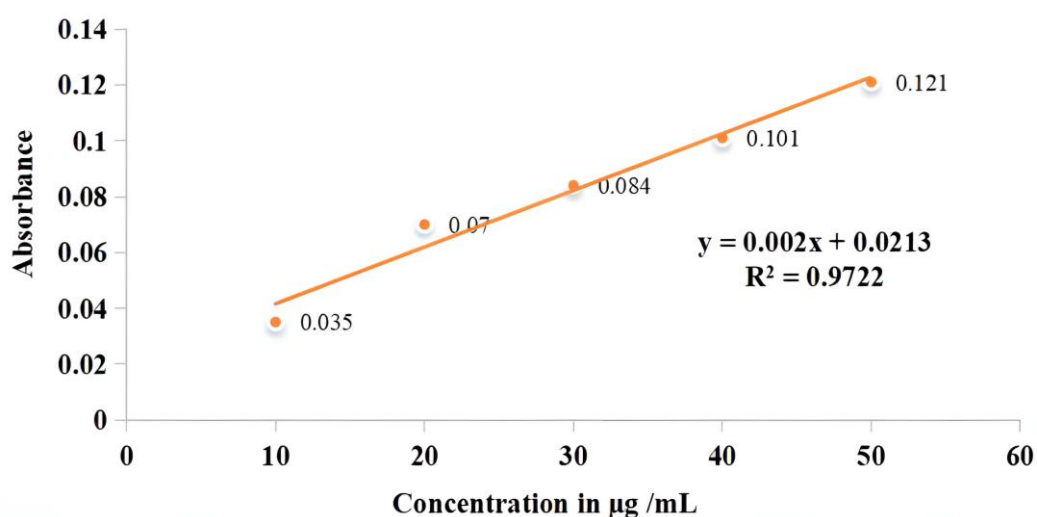


Figure 1: Standard curve of rutin

Total Tannin Content

The total tannin content varied considerably among the samples examined. The leaf extract exhibited the highest tannin concentration at 98.0 µg/mL, indicating a rich presence of polyphenolic compounds. This was followed by the stem (38.5 µg/mL) and the flower (28.0 µg/mL), while tannins were undetectable in the fruit extract. These findings suggest that the leaves of *P. daemia* may serve as a potent source of antioxidant and antimicrobial agents, aligning with their traditional usage in treating inflammatory and infectious conditions, **tannic acid used as reference standard** (Fig. 2).

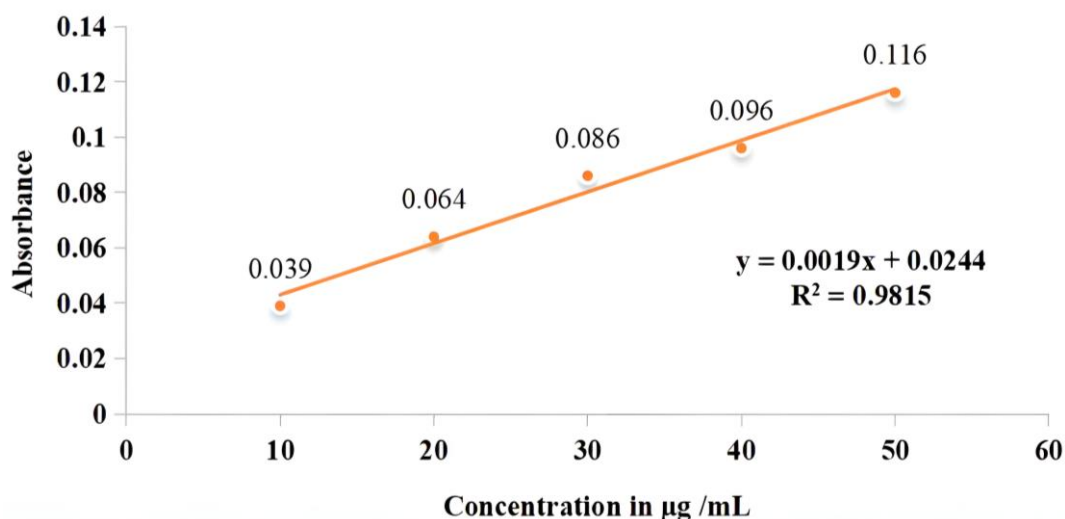


Figure 2: Standard curve of tannic acid

Table 3. Total Tannin Content in Different Parts of *Pergularia daemia*

Sample of <i>P. daemia</i>	Total Tannin Concentration (µg/ml)
Leaf	98
Stem	38.5
Flower	28

3.2. GC-MS Analysis

The GC-MS analysis of ethanol extracts from different parts of *Pergularia daemia*—flower, fruit, leaf, and stem revealed a total of 19, 14, 14, and 11 peaks, respectively. The identified compounds varied qualitatively and quantitatively among the plant parts. The stem extract exhibited the highest number of peaks (19). Major components included n-hexadecanoic acid (33.01%) and 9,12,15-octadecatrienoic acid (17.46%). Other compounds detected were 5-hydroxymethylfurfural (1.77%), 1,2,3-propanetriol (4.88%), diethyl phthalate (3.11%), and 1,2-benzenedicarboxylic acid (1.60%). In the leaf extract, 14 peaks were detected. The dominant compounds were 9,12,15-octadecatrienoic acid (48.33%) and n-hexadecanoic acid (22.27%). Other components included tetradecanoic acid (3.57%), octadecanoic acid (2.17%), and various hydrocarbons such as dodecane and tetradecane. Loliolide, a monoterpenoid lactone, was also present. In the flower extract, major constituents included α -amyrin

(41.54%), olean-12-en-3-ol, acetate (3 β)- (24.74%), lupeol (16.16%), and stigmasterol (9.50%). Minor compounds detected were n-hexadecanoic acid (0.59%), 1,2,3-propanetriol (0.94% and 0.49%), dodecane (0.28%), proceroside (0.31%), germanicol acetate (4.32%), and (2E)-1-(Methoxymethoxy)-2-octene (1.15%). The fruit extract showed the presence of 14 compounds, with notable peaks including n-hexadecanoic acid (13.12%), 9,12,15-octadecatrienoic acid (17.10%), 1,2,3-propanetriol (2.44%), and diethyl phthalate (3.27%). Hydrocarbons such as dodecane, tetradecane, and hexadecane were also identified. Additionally, 1,2-benzenedicarboxylic acid and methyl oleate were present in low abundance (Figure 3-6; Table 4-7). Each plant part displayed a unique phytochemical fingerprint, with certain compounds common across multiple parts and others uniquely present in specific tissues (Table 8).

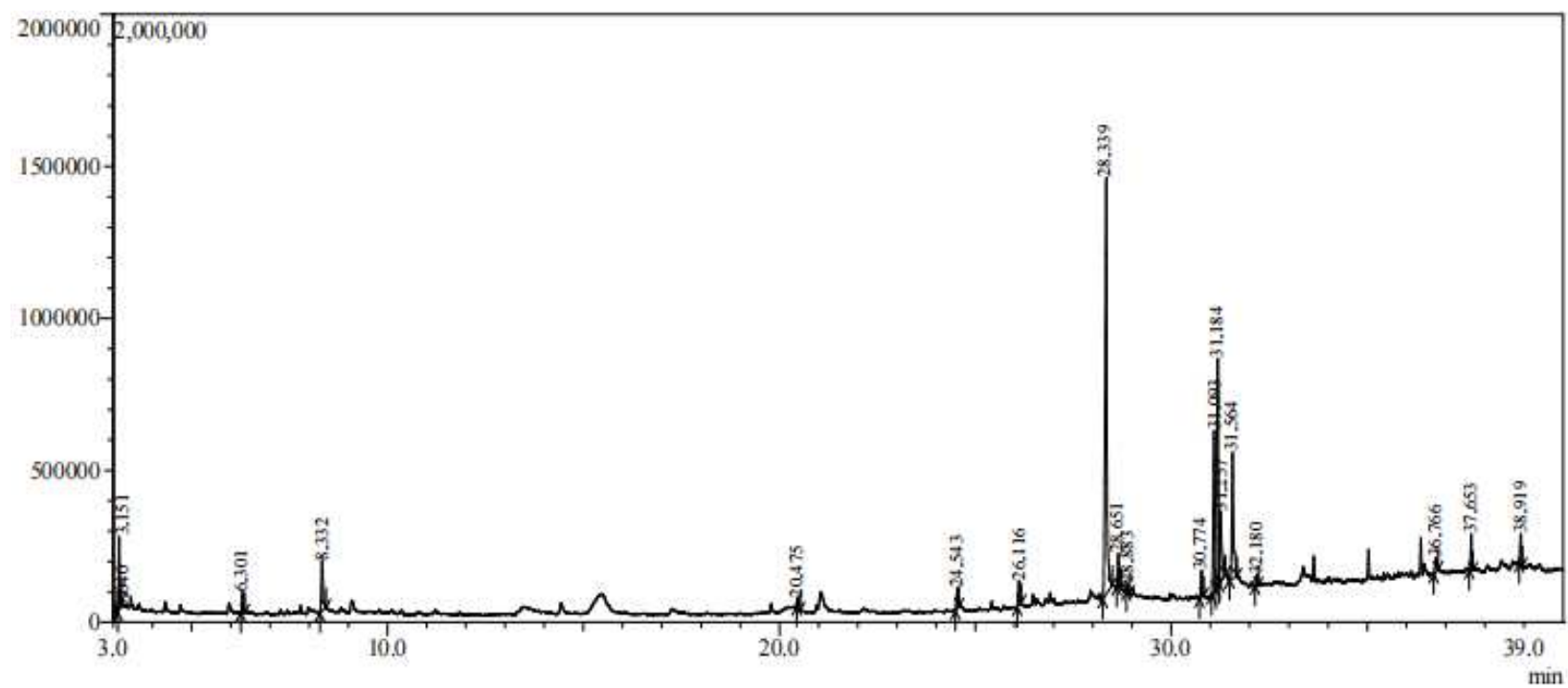
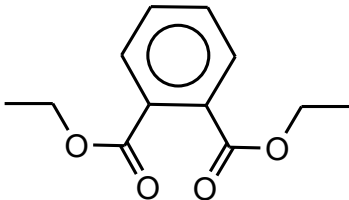
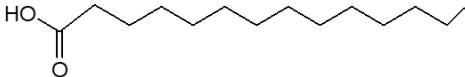
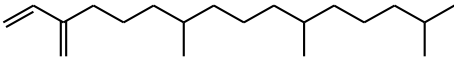
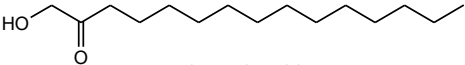
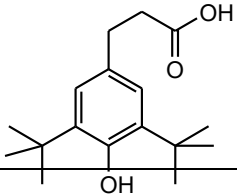
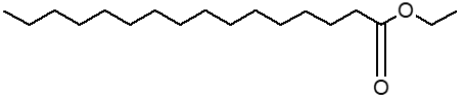
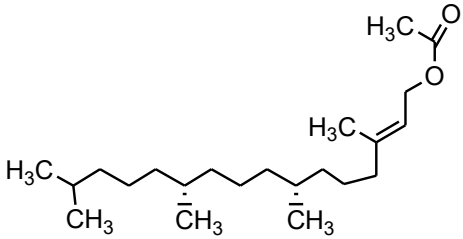
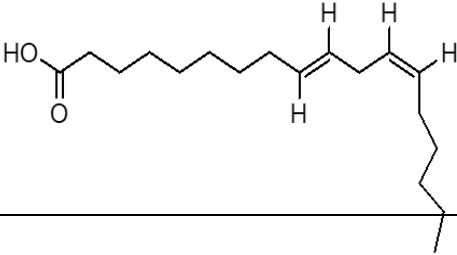


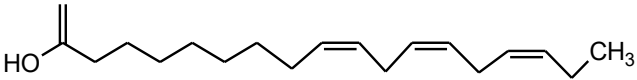
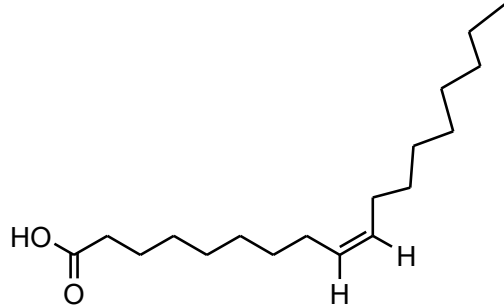
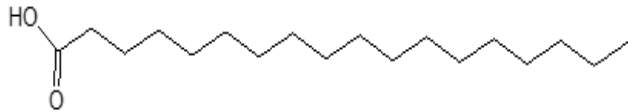
Figure 3: GC-MS chromatogram of ethanolic stem extract of *Pergularia daemia*, displaying identified phytochemical peaks.

Table 4: GC-MS peak table of stem extract showing compounds, molecular formulae, and structures.

S. No.	Name	Molecular Formula	Chemical structure
1	3,4,4-D3-Trans-3,5-Dihydroxy-Cyclopentene	C ₅ H ₅ D ₃ O ₂	
2	1,2,3-Propanetriol	C ₃ H ₈ O ₃	
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	
4	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	

5	Diethyl Phthalate	C ₁₂ H ₁₄ O ₂	
6	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	
7	Neophytadiene	C ₂₀ H ₃₈	
8	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	
9	3,5-di-tert-Butyl-4-hydroxyphenylpropionic acid	C ₁₇ H ₂₆ O ₃	

10	Hexadecanoic acid, ethyl ester	C18H36O2	
11	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]-	C22H42O2	
12	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	

13	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	
14	Oleic acid	C ₁₈ H ₃₄ O ₂	
15	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	
16	Heptatriacontane	C ₃₇ H ₇₆	

17	Nonacosanal	C ₂₉ H ₅₈ O	
18	Heptatriacontane	C ₃₇ H ₇₆	
19	Tetrapentacontane	C ₅₄ H ₁₁₀	

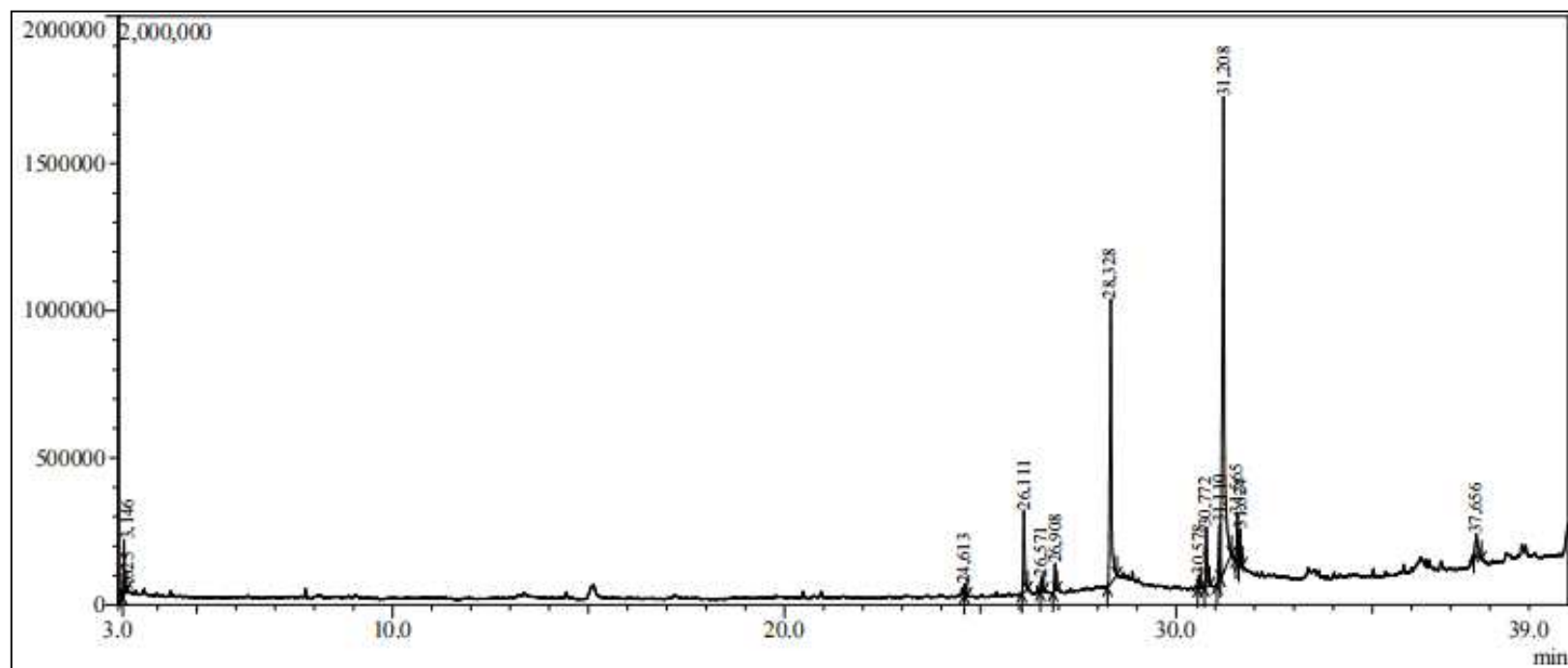
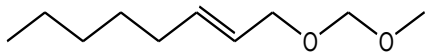
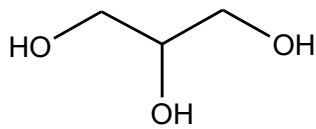
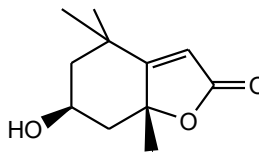
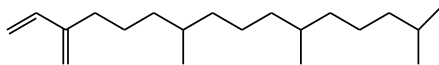
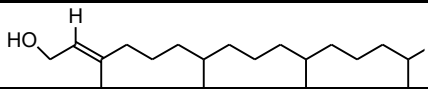
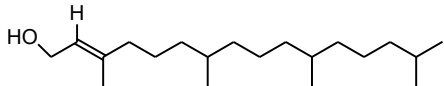
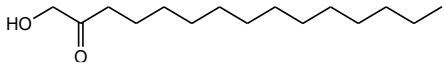
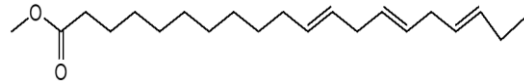
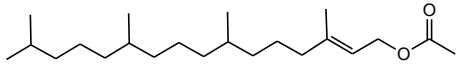
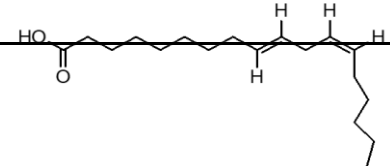
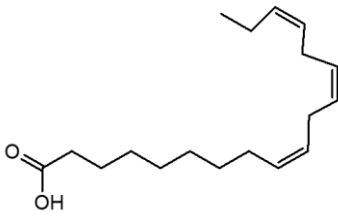
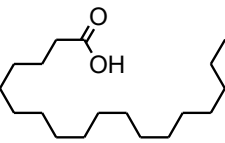
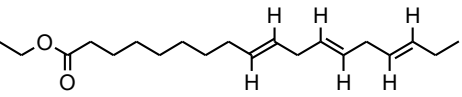


Figure 3: GC-MS chromatogram of ethanolic leaf extract of *Pergularia daemia*, illustrating the presence of major metabolites.

Table 5: GC-MS profile of *Pergularia daemia* leaf extract, listing phytochemicals with molecular formula and structural information.

S. No.	Name	Molecular Formula	Chemical Structure
1	2-Octene, 1-(methoxymethoxy)-, (E)-	$C_{10}H_{20}O$	
2	Glycerin	$C_3H_8O_3$	
3	Loliolide	$C_{11}H_{16}O_3$	
4	Neophytadiene	$C_{20}H_{38}$	
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	

6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	
8	11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	
9	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	$C_{22}H_{42}O_2$	
10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	

11	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	
12	Octadecanoic acid	$C_{18}H_{36}O_2$	
13	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	
14	Iron Iodide Complex I	FeI_3	I^- Fe^{+++}

I^- I^-

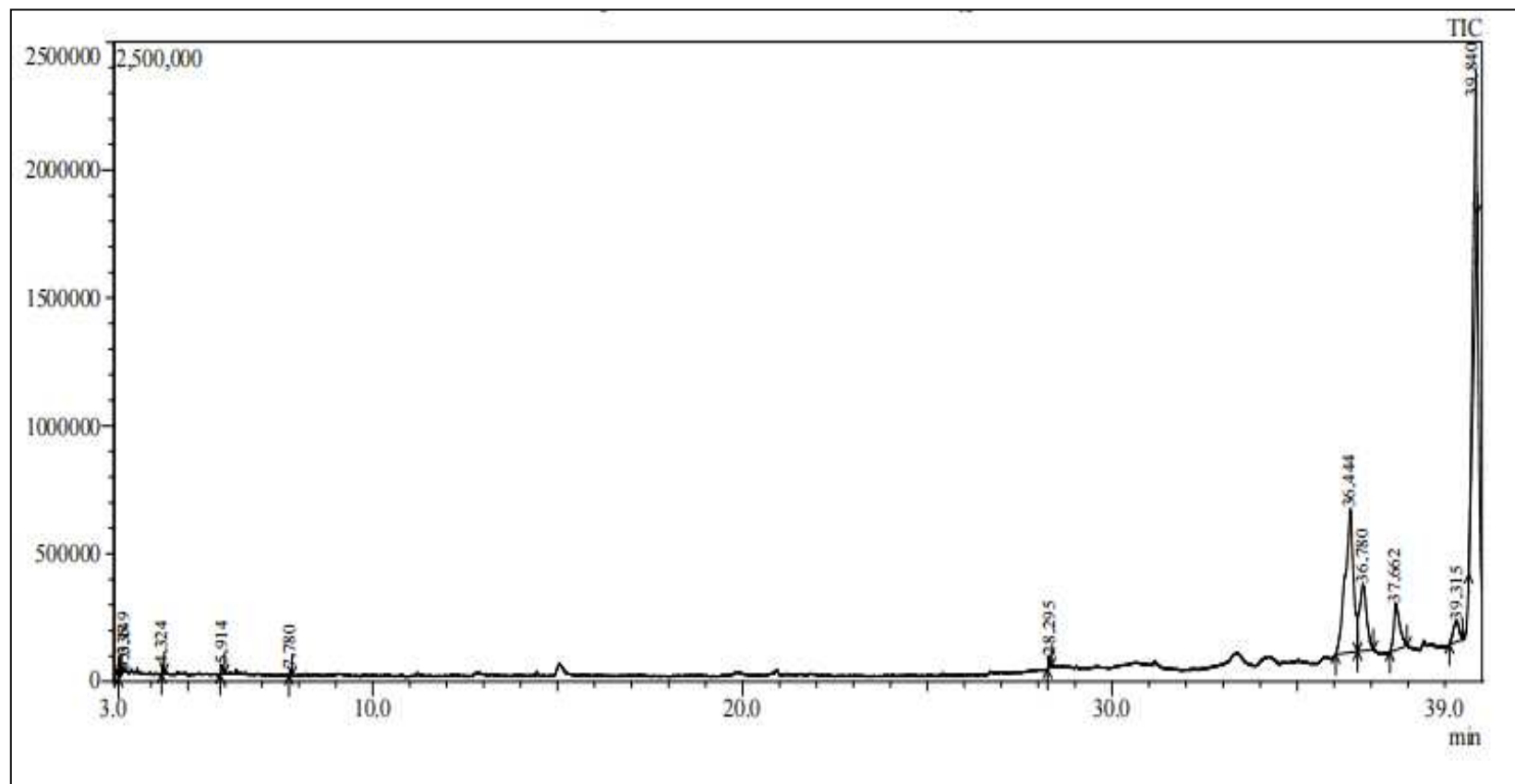
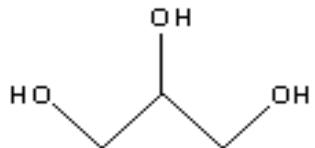
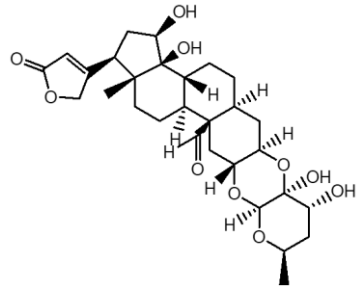
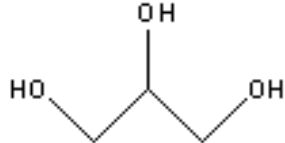
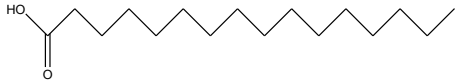
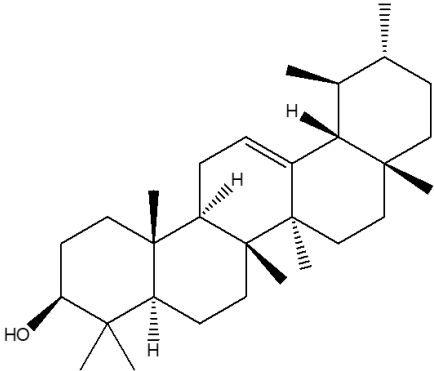
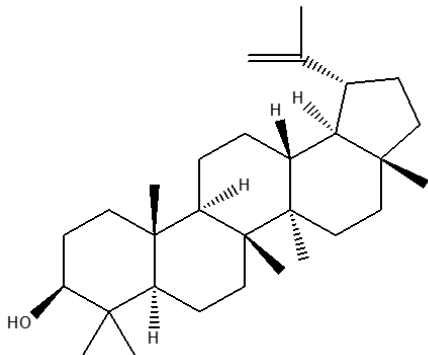
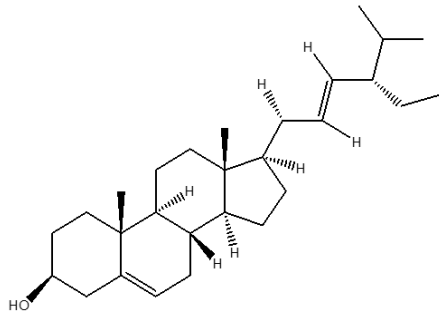
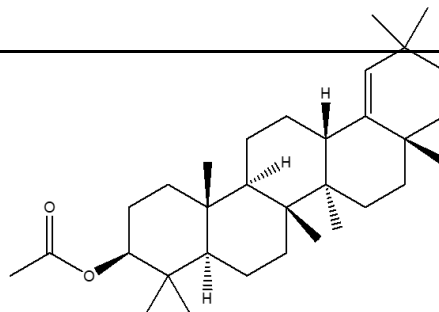


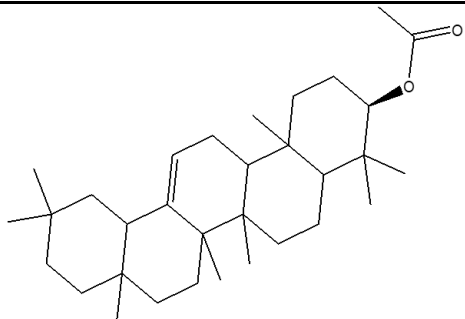
Figure 4: GC-MS chromatogram of ethanolic flower extract of *Pergularia daemia*, showing prominent triterpenoids and sterols.

Table 6: GC-MS data for *Pergularia daemia* flower extract, detailing triterpenoids, sterols, and other constituents

S.No.	Name	Molecular Formula	Chemical Structure
1	(2E)-1-(Methoxymethoxy)-2-Octene	$C_{10}H_{20}O_2$	
2	1,2,3-Propanetriol	$C_3H_8O_3$	
3	Proceroside	$C_{29}H_{40}O_{10}$	

4	1,2,3-Propanetriol	$C_3H_8O_3$	
5	Dodecane	$C_{12}H_{26}$	
6	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	
7	α -Amyrin	$C_{30}H_{50}O$	

8	Lupeol	$C_{30}H_{50}O$	
9	Stigmasterol	$C_{29}H_{48}O$	
10	Germanicol acetate	$C_{32}H_{52}O_2$	

11	Olean-12-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$	

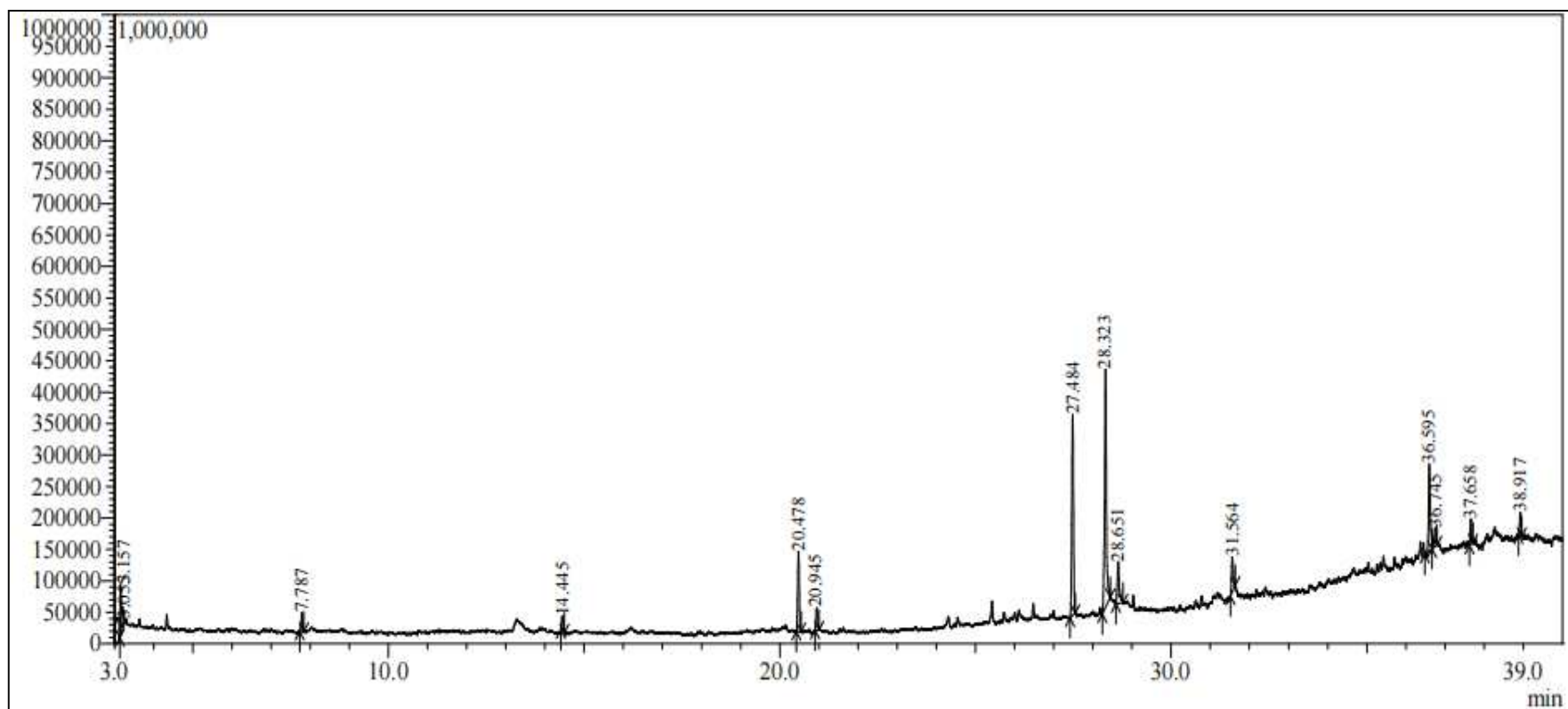
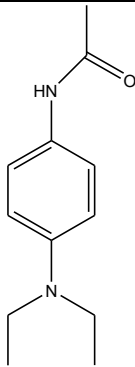
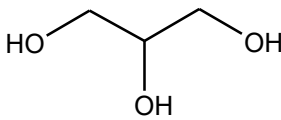
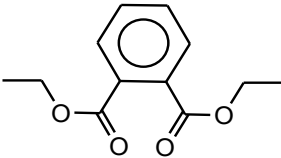
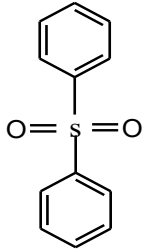
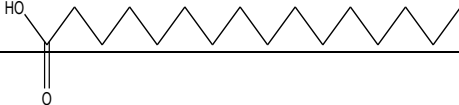
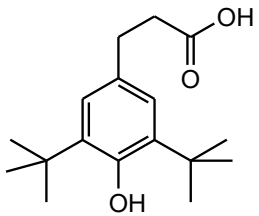
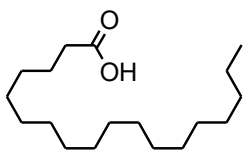
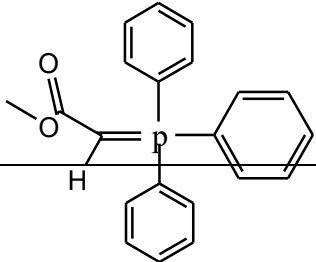


Figure 5: GC-MS chromatogram of ethanolic fruit extract of *Pergularia daemia*, indicating diverse bioactive constituents.

Table 7: Phytochemical composition of *Pergularia daemia* fruit extract based on GC-MS analysis, with molecular formula and structures

S.No.	Name	Molecular Formula	Chemical Structure
1	N-(4-(Diethylamino)phenyl)acetamide	$C_{12}H_{18}N_2O$	
2	Glycerin	$C_3H_8O_3$	
3	Dodecane	$C_{12}H_{26}$	
4	Tetradecane	$C_{14}H_{30}$	

5	Diethyl Phthalate	$C_{12}H_{14}O_4$	
6	Hexadecane	$C_{16}H_{34}$	
7	Diphenyl sulfone	$C_{12}H_{10}O_2S$	 Diphenyl sulfone
8	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	

9	3,5-di-tert-Butyl-4-hydroxyphenylpropionic acid	$C_{17}H_{26}O_3$	
10	Octadecanoic acid	$C_{18}H_{36}O_2$	
11	Acetic acid, (triphenylphosphoranylidene)-, methyl ester	$C_{21}H_{19}O_2P$	

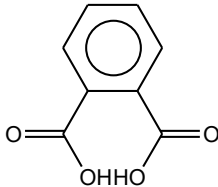
12	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	
13	Tetrapentacontane	$C_{54}H_{110}$	
14	Tetrapentacontane	$C_{54}H_{110}$	

Table 8: Quantitative GC-MS Highlights of Major Bioactive Compounds Identified in Different Parts of *Pergularia daemia*

Plant Part	Major Compound	Area %	Functional Properties
Stem	n-Hexadecanoic acid	33.01%	Antioxidant, antimicrobial
Leaf	9,12,15-Octadecatrienoic acid	48.33%	Antioxidant, cardioprotective
Flower	α -Amyrin	41.54%	Anti-inflammatory, hepatoprotective, anticancer
Fruit	n-Hexadecanoic acid	28.63%	Antioxidant, antimicrobial

3.3. In Silico Docking Analysis of α -Amyrin

Molecular docking of α -Amyrin with the human transforming growth factor protein (PDB ID: 1TGK) yielded a binding affinity of -6.9 kcal/mol, indicating a moderate yet stable interaction. The docking results identified key amino acid residues in the active site involved in binding, primarily through hydrophobic and van der Waals interactions. No hydrogen bonding was detected. The 2D interaction plot illustrated the amino acid residues contributing to the binding through non-polar interactions (Figure 6), while 3D visualization confirmed that α -Amyrin is well accommodated within the binding pocket of the protein (Figure 7), suggesting spatial compatibility that supports the docking outcome.

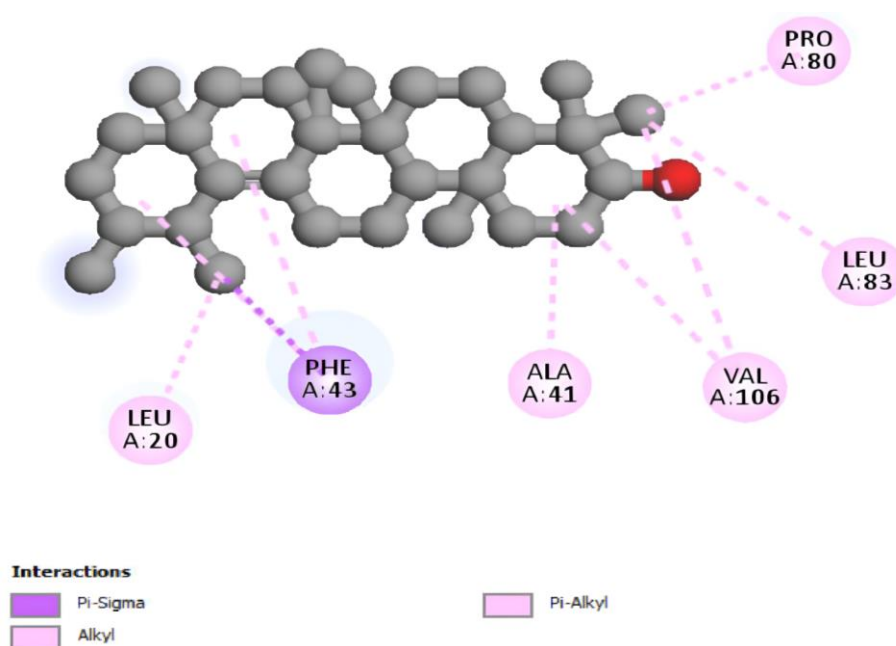


Figure 6: 2D interaction diagram of α -Amyrin bound to the active site of 1TGK with binding energy -6.9 kcal/mol.

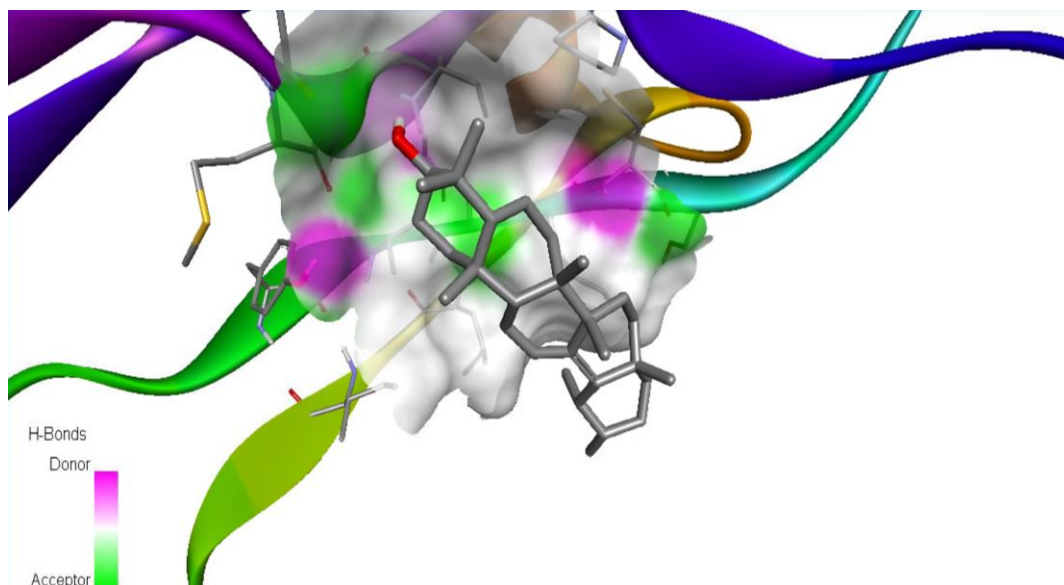


Figure 7: 3D docking pose of α -Amyrin within the 1TGK binding pocket, showing favorable orientation and surface complementarity.

4. Discussion

The phytochemical investigation of *Pergularia daemia* revealed a diverse array of bioactive compounds distributed selectively across different plant parts—leaf, stem, flower, and fruit—highlighting its therapeutic potential and supporting its ethnomedicinal relevance. The qualitative analysis confirmed the presence of flavonoids in all four parts, substantiating their role as key phytoconstituents. Flavonoids are well-documented for their antioxidant, anti-inflammatory, and antimicrobial properties [32], and their presence in *P. daemia* was previously reported by [33], who linked them to antidiabetic and hepatoprotective effects in methanolic leaf extracts. Tannins were notably abundant in the leaf (98 $\mu\text{g/mL}$), with moderate levels in the stem and flower. Known for their astringent, antimicrobial, and wound-healing properties [34], tannins support traditional uses of *P. daemia* in treating skin

ailments and gastrointestinal disorders [35]. Glycosides, detected in the stem and flower, are associated with cardioprotective and anti-inflammatory activities. Interestingly, alkaloids were restricted to the stem, suggesting tissue-specific biosynthesis. This aligns with findings by [36], who documented the analgesic and anthelmintic properties of stem-derived alkaloids. Saponins, present in the leaf and stem, are known for their immunostimulatory, hypocholesterolemic, and antimicrobial properties [37], further supporting the plant's ethnobotanical use in boosting immunity.

Conversely, the fruit exhibited limited phytochemical content, with low flavonoid levels (12 µg/mL) and no detectable tannins, alkaloids, or terpenoids, suggesting it may play a secondary pharmacological role unless further studied. GC-MS analysis of ethanolic extracts from the leaf, stem, flower, and fruit provided a deeper insight into the metabolite diversity of *P. daemia*. Fatty acids such as palmitic acid (n-hexadecanoic acid) and stearic acid (octadecanoic acid) were consistently identified in all extracts, with high concentrations in the leaf (22.27%) and stem (33.01%). These compounds possess antimicrobial, antioxidant, and anti-inflammatory activities [38] and likely contribute to the plant's defensive and structural roles. The presence of 9,12,15-octadecatrienoic acid, a polyunsaturated fatty acid, in high amounts in the leaf (48.33%) and stem (17.46%) supports its role in oxidative stress reduction. Pallavi et al. (2020) [39] similarly reported bioactive lipids in *P. daemia* leaves. The flower extract was particularly rich in triterpenoids such as α -amyrin (41.54%), lupeol (16.16%), and olean-12-en-3-ol, acetate (24.74%), compounds known for their anti-inflammatory, hepatoprotective, and anticancer potential. Additionally, the detection of stigmasterol (9.50%) and germanicol acetate (4.32%) highlights the flower's enriched sterol content, supporting its use in traditional remedies for liver and

inflammatory conditions. Glycerol, found in the flower, fruit, and stem extracts, may play a role in osmotic regulation and stress adaptation. Hydrocarbons such as dodecane, tetradecane, and hexadecane were predominantly identified in the leaf and fruit and are known to function as antimicrobial and pesticidal agents [40]. Though compounds like diethyl phthalate and 1,2-benzenedicarboxylic acid are often considered environmental contaminants, some studies suggest they may have plant-derived antimicrobial roles [41], though further investigation is warranted. Notably, unique compounds such as Proceroside (a cardiac glycoside) in the flower and Loliolide (an antioxidant monoterpene lactone) in the leaf indicate specialized therapeutic potentials. The stem extract contained 5-hydroxymethylfurfural, an antioxidant and anticancer compound, reinforcing the medicinal relevance of this tissue. These findings collectively align with documented the pharmacological efficacy and phytochemical richness of *P. daemia*. The organ-specific metabolite distribution observed in this study underscores the plant's potential for targeted extraction and application in herbal formulations [44]. Among the four major phytoconstituents identified through GC-MS analysis of the leaf, stem, flower, and fruit extracts of *Pergularia daemia*, α -Amyrin was selected for molecular docking due to its consistently high peak intensity and its well-established pharmacological relevance. α -Amyrin is a pentacyclic triterpenoid widely known for its anti-inflammatory, analgesic, and anticancer activities, which supports its selection as a bioactive candidate for in silico interaction studies. The docking results revealed a binding energy of -6.9 kcal/mol, indicating a moderate yet stable interaction between α -Amyrin and the human Transforming Growth Factor-beta (TGF- β) receptor (PDB ID: 1TGK). The interaction was predominantly governed by hydrophobic and van der Waals forces, consistent with the compound's non-polar, lipophilic nature. The

absence of hydrogen bonding further emphasizes the role of hydrophobic interactions in mediating the stability of the ligand–receptor complex. Furthermore, the 3D binding visualization confirmed that α -Amyrin fits well within the binding cavity of 1TGK, highlighting spatial complementarity and reinforcing its potential as a modulator of the TGF- β pathway. Since this signaling pathway plays a crucial role in inflammation, fibrosis, and cancer progression, the interaction of α -Amyrin with 1TGK may offer a novel therapeutic avenue. These findings provide computational evidence supporting the pharmacological promise of α -Amyrin. However, to validate its therapeutic efficacy, in vitro and in vivo studies are essential. Such studies should aim to explore the compound's mechanism of action, specificity, and safety profile in biological systems.

5. Conclusion

This study confirms the phytochemical diversity of *Pergularia daemia*, revealing key bioactive compounds such as α -Amyrin, lupeol, n-hexadecanoic acid, and 9,12,15-octadecatrienoic acid across various plant parts. GC-MS profiling and qualitative analysis highlighted the therapeutic relevance of flavonoids, terpenoids, and fatty acids. Molecular docking of α -Amyrin against the 1TGK protein demonstrated notable binding affinity (-6.9 kcal/mol), suggesting its potential role in modulating TGF- β signaling. These findings validate the plant's traditional applications and underscore its potential for development into phytotherapeutic agents targeting inflammation and liver-related disorders. Further in vivo studies are warranted to explore its efficacy and safety.

Declaration

Conflict of interest

Authors don't have potential conflict of interest among them

Authors contribution

RA: Writing original draft, reviewed & editing, finalization, conceptualization, and formal analysis, **SS:** Work design, editing, finalization, conceptualization, visualizations, **HR:** Formal analysis, visualizations, reviewed & editing, **RK:** finalization, visualizations, conceptualization, reviewed & editing, **SS:** Formal analysis, visualizations, reviewed & editing, **BC:** visualizations, conceptualization, reviewed & editing. **AP:** finalization, visualizations, conceptualization, reviewed & editing, **SK:** finalization, conceptualization, and formal analysis, **SM, CP, and AA:** Formal analysis, visualizations, conceptualization, reviewed & editing. All the authors gave final approval of the version to be submitted.

Data availability

Necessary data included apart from that for further clarifications need to contact corresponding author.

Clinical trail

Not Applicable

Funding

This article is derived from a research grant funded by the Research, Development, and Innovation Authority (RDIA)-Kingdom of Saudi Arabia with grant number (13445-Tabuk-2023-UT-R-3-1-SE).

References

- [1] Chandak, R. R., & Dighe, N. S. (2019). A review on phytochemical & pharmacological profile of *Pergularia daemia* linn. *Journal of Drug Delivery and Therapeutics*, 9, 809-814.
- [2] Ananth, D. A., Deviram, G., Mahalakshmi, V., & Bharathi, V. R. (2021). Active status on phytochemistry and pharmacology of *Pergularia daemia* Forsk. (Trellis-vine): a review. *Clinical Phytoscience*, 7, 1-13.

- [3] Kandeda, A. K., Moto, F. C. O., Ayissi, R. E. M., Omam, J. P. O., Ojong, L., & Bum, E. N. (2021). Pergularia daemia hydro-ethanolic extract protects against pentylenetetrazole kindling-induced seizures, oxidative stress, and neuroinflammation in mice. *Journal of Ethnopharmacology*, 279, 114338.
- [4] Yakubu, R., Jibril, F. M., Lukman, A., & Sheikh, F. Research Article Trends for Antioxidant Power of Phytochemicals from Pergularia tomentosa L.(Asclepiadaceae) Whole Plant.
- [5] Patra, M., Giri, S. K., & Chowdhury, S. (2021). Phytochemical screening and galactagogic attributes of Pergularia daemia (Forssk.) Chiov. leaf extract in lactating mice (Mus musculus). *Herba Polonica*, 67(4), 32-41.
- [6] Nithyatharani, R., & Kavitha, U. (2018). Phytochemical studies on the leaves of Pergularia daemia collected from Villupuram District, Tamil Nadu, India. *IOSR Journal of Pharmacy*, 8(1), 9-12.
- [7] Vijayasanthi, M., Doss, A., & Kumar, K. A. (2018). Evaluation of in-vitro anti-inflammatory and antioxidant activity of pergularia daemia linn. *World J. Pharm. Pharm. Sci.*, 7(7), 524-531.
- [8] Hase, V., & Nasreen, S. (2016). Pharmacognostical aspects of Pergularia daemia Leaves. *International Journal of Applied Research*, 2, 296-300.
- [9] Anooj, S. M., Betty, T., Malini, R. P., Vasini, V., & Sumathi, P. (2022). Phytochemical and antimicrobial investigations on the aerial plant parts of pergularia daemia forsk.(asclepiadaceae): a perennial twinning herb. *Kongunadu Research Journal*, 9(2), 68-74.
- [10] Bhusari, S., Bhokare, S. G., Nikam, K. D., Chaudhary, A. N., & Wakte, P. S. (2018). Pharmacognostic and Phytochemical investigation of stems of Pergularia daemia. *Asian Journal of Pharmacy and Pharmacology*, 4(4), 500-504.

- [11]Nithyatharani, R., & Kavitha, U. S. (2018). Qualitative and quantitative analysis of the stem of *Pergularia daemia*.
- [12]Bhaskar, V. H., & Balakrishnan, N. (2010). Protective effects of *Pergularia daemia* roots against paracetamol and carbon tetrachloride-induced hepatotoxicity in rats. *Pharmaceutical Biology*, 48(11), 1265-1272.
- [13]Nikajoo, L. T. (2009). Analgesic activity of aqueous and alcohol root extracts of *Pergularia daemia* (forsk.) chiov. *Int J Pharm Pharm Sci*, 1(Suppl 1), 33-37.
- [14]Nandhakumar Palraj, V. S. V., Chidambaranathan, N., & Vijayan, M. (2019). Evaluation of anti-inflammatory and antinociceptive effects of latex from *Pergularia daemia* in experimental animal models. *Asian Journal of Pharmacy and Pharmacology*, 5(5), 1000-1009.
- [15]Patil, C. D., Borase, H. P., Patil, S. V., Salunkhe, R. B., & Salunke, B. K. (2012). Larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* plant latex against *Aedes aegypti* and *Anopheles stephensi* and nontarget fish *Poecillia reticulata*. *Parasitology research*, 111, 555-562.
- [16]Vijayakumar, A. S., Ramya, B., Vijikumar, S., Ramanathan, K., & Ramya, M. B. Structural characterization and Antifungal activity in Crude Latex Extracts with Drug Designing using *Calotropis procera* L., *Pergularia daemia* L., and *Sarcostemma intermedium* Decne.
- [17]Brahmam, P., Sunita, K., & Babu, B. H. (2018). Phytochemical investigation of plants *Limonia acidissima* (L.) and *Pergularia daemia* (L.) from Prakasam district, Andhra Pradesh, India. *Eur. J. Biomed. Pharm. Sci*, 5(5), 977-983.
- [18]Asmaey, M. A. (2024). Utilizing GC–MS and UPLC–MS for rapid metabolomic analysis in medicinal plants. In *Propagation to Pharmacopeia* (pp. 361-378). CRC Press.

- [19] Barthwal, R., & Mahar, R. (2024). Exploring the significance, extraction, and characterization of plant-derived secondary metabolites in complex mixtures. *Metabolites*, 14(2), 119.
- [20] Lobo, M. G., Hounsome, N., & Hounsome, B. (2018). Biochemistry of vegetables: secondary metabolites in vegetables—terpenoids, phenolics, alkaloids, and sulfur-containing compounds. *Handbook of vegetables and vegetable processing*, 47-82.
- [21] Yau, W. P., Goh, C. H., & Koh, H. L. (2015). Quality control and quality assurance of phytomedicines: Key considerations, methods, and analytical challenges. *Phytotherapies: efficacy, safety, and regulation*, 18-48.
- [22] Beale, D. J., Pinu, F. R., Kouremenos, K. A., Poojary, M. M., Narayana, V. K., Boughton, B. A., ... & Dias, D. A. (2018). Review of recent developments in GC–MS approaches to metabolomics-based research. *Metabolomics*, 14, 1-31.
- [23] Arjun P. 2011. Investigations on Micropropagation and Isolation of Active Compounds from *Tribulus terrestris* Linn. Ph.D thesis, Faculty of Sciences, University of Madras, Chennai, India.
- [24] Azhagu Madhavan S., Arjun P, Kedari G.S.R., Vinay Kumar, 2025. Identification of Bio-Active Compounds and Biochemical Characterization of Green Synthesized of Silver Nanoparticles (AgNPs) against Selected Uropathogenic Antimicrobial Activity. *Journal of Inorganic and Organometallic Polymers and Materials*, 35(4):2904-2920.
- [25] Azhagu Madhavan S., Arjun P. 2024. Characterization of silver nanoparticles (AgNPs) synthesized using polyphenolic compounds from *Phyllanthus emblica* L. and their impact on cytotoxicity in human cell lines. *Carbohydrate Polymer Technologies and Applications*. 8, 100535.

- [26] Sumathy R., Azhagu Madhavan S., Arjun P., Pankaj Kumar C. 2024. Nanomaterial (AgNPs) Synthesis Using *Calotropis gigantea* Extract, Characterization and Biological Application in Antioxidant and Antibacterial Activity. *Journal of Inorganic and Organometallic Polymers and Materials*. 34(9):4005-4021.
- [27] Gurupriya, S., Cathrine, L., & Ramesh, J. (2017). Qualitative and quantitative phytochemical analysis of Simarouba glauca leaf extract. *Int. J. Res. Appl. Sci. Eng. Technol*, 5(11), 475-479.
- [28] Sharma, P., & Modi, N. (2023). Qualitative and quantitative phytochemical screening and antioxidant potential of different extracts of *Opuntia ficus indica* fruits. In *Biological Forum—An International Journal* (Vol. 15, No. 4, pp. 694-701).
- [29] Arjun P., Semwal, D.K., Semwal, R.B., M. Malaisamy, C. Sivaraj and S. Vijayakumar. 2017. Total Phenolic Content, Volatile Constituents and Antioxidative Effect of *Coriandrum sativum*, *Murraya koenigii* and *Mentha arvensis*. *The Natural Products Journal*, 7(1): 65-74. doi:10.2174/2210315506666161121104251
- [30] National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 73170, alpha-Amyrin. Retrieved May 6, 2025 from <https://pubchem.ncbi.nlm.nih.gov/compound/alpha-Amyrin>.
- [31] Shamsi, A., Khan, M. S., Yadav, D. K., Shahwan, M., Furkan, M., & Khan, R. H. (2024). Structure-based drug-development study against fibroblast growth factor receptor 2: molecular docking and Molecular dynamics simulation approaches. *Scientific Reports*, 14(1), 19439.

- [32] Rondón, M., García, I., Cornejo, X., Rojas, J., & Terán, W. (2015). Phytochemical screening and antioxidant activity of seven medicinal plants species from Ecuador. *Pharmacology Online*, 3, 19-28.
- [33] Doss, A., Tresina, P. S., & Mohan, V. R. Antidiabetic and Antiinflammatory Activity of Methanol and Aqueous Extracts of *Pergularia daemia* (Forssk.) Chiov. *Advances and Trends in Biotechnology and Genetics*, 85.
- [34] Ignacimuthu, S., Pavunraj, M., Duraipandiyar, V., Raja, N., & Muthu, C. (2009). Antibacterial activity of a novel quinone from the leaves of *Pergularia daemia* (Forsk.), a traditional medicinal plant. *Asian Journal of Traditional Medicines*, 4(1), 36-40.
- [35] James, O., Unekworo, E. G., & Ojochenemi, A. A. (2011). Assessment of biological activities: A comparison of *Pergularia daemia* and *Jatropha curcas* leaf extracts. *British Biotechnology Journal*, 1(3), 85-100.
- [36] Sutar, N. G., & Pal, S. C. (2015). Finger printing analysis of the flavonoid from leaves *Pergularia daemia* Forsk using HPTLC analysis. *J Pharmacog Phytochem*, 3(5), 157-161.
- [37] Sparg, S., Light, M. E., & Van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of ethnopharmacology*, 94(2-3), 219-243.
- [38] Sridevi, G., Prema, S., Sekar, S., & Sembulingam, K. (2014). Phytochemical analysis of *Pergularia daemia* for its bioactive components through gas chromatographic mass spectrometry (GCMS). *IOSR Journal of Pharmacy*, 4(5), 41-46.
- [39] Pallavi, A., Nagababu, P., Hari, B. B., & Brahmaji, R. P. (2020). Separation and Identification of New Phytocomponents in Methanolic Extract of Leaves of

Hairknot Plant (P. daemia) by GC-MS Analysis. *Oriental Journal of Chemistry*, 36(6), 1229.

- [40] Dosumu, O. O., Ajetumobi, O. O., Omole, O. A., & Onocha, P. A. (2019). Phytochemical composition and antioxidant and antimicrobial activities of *Pergularia daemia*. *Journal of Medicinal Plants for Economic Development*, 3(1), 1-8.
- [41] Doss, A., & Anand, S. P. (2013). Antimicrobial activity of *Hygrophila auriculata* (Schumach.) Heine and *Pergularia daemia* Linn. *African Journal of Plant Science*, 7(4), 137-142.
- [42] Poornima, R., & Averal, H. I. (2018). In vivo antidiabetic and antilipidemic activities of *Pergularia daemia* on pcos induced rats.
- [43] Mickymaray, S., Al Aboody, M. S., Rath, P. K., Annamalai, P., & Nooruddin, T. (2016). Screening and antibacterial efficacy of selected Indian medicinal plants. *Asian Pacific Journal of Tropical Biomedicine*, 6(3), 185-191.
- [44] Vaithyanathan, V., & Mirunalini, S. (2014). Quantitative variation of bioactive phyto compounds in ethyl acetate and methanol extracts of *Pergularia daemia* (Forsk.) Chiov. *Journal of Biomedical Research*, 29(2), 169.