*Int J Adv Life Sci Res. Volume 9(3) 151-164*
https://doi.org/10.31632/ijalsr.2026.v09i03.012

**International Journal of Advancement in Life Sciences Research**

Online ISSN: 2581-4877

*Journal homepage* http://ijalsr.org

Original Article

# Phytochemical Profile and Evaluation of Biological Properties of *Plectranthus vettiveroides* Root Extract – (Vilamichai Root) An Extensive *in vitro* Study

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**Abstract**

**Background:** *Plectranthus vettiveroides* is an aromatic medicinal herb traditionally used in Ayurvedic and Siddha medicine. Although previous studies have reported its antioxidant, antidiabetic, and anticancer properties, its phytochemical profile and broad biological activities remain incompletely characterised. In parts of South Asia, the roots (Vilamichai root) are traditionally soaked in drinking water for health benefits, but scientific evidence supporting this practice is limited. **Objectives:** This study aimed to characterise the phytochemical constituents of *Plectranthus vettiveroides roots and evaluate their biological activities using multiple in vitro* assays. **Methods:** Ethanolic root extracts were subjected to qualitative and quantitative phytochemical analyses. Their antidiabetic, anti-inflammatory, antilipidemic, antioxidant, antiurolithiatic, immunomodulatory, cytotoxic, and antibacterial activities were assessed using established *in vitro methods.* **Results:** Qualitative analysis demonstrated tannins, saponins, flavonoids, steroids, alkaloids, and anthraquinones. The extract contained high total phenolic (223.57 mg GAE/g) and flavonoid (173.19 mg QE/g) contents. It inhibited α-amylase (87.66%) and α-glucosidase (85.75%) activities, suppressed protein denaturation (88.22%), and exhibited strong antioxidant activity across multiple assays. The extract also inhibited calcium oxalate crystallisation, enhanced phagocytic activity, showed cytotoxicity against HeLa cells ($IC_{50}$ 267.44 µg/mL), and demonstrated antibacterial activity against *Vibrio parahaemolyticus* and *Vibrio harveyi.* **Conclusion:** *Plectranthus vettiveroides* root extract possesses diverse bioactive phytochemicals with promising antidiabetic, antioxidant, anti-inflammatory, antiurolithiatic, immunomodulatory, cytotoxic, and antibacterial properties. These findings support its traditional use as a health-promoting herbal preparation and warrant further *in vivo* and clinical studies to validate its therapeutic potential.

***Keywords:*** *Anti-Diabetic, Anti-Inflammatory, Antioxidant, Anti-Bacterial, Anti- Lipidemic; Vilamichai Ver*

*Received on :25th October 2025; Revised version received on :13th June 2026; Accepted: 15th June 2026*

## Introduction

The medicinal potential of plant-derived compounds has garnered increasing attention recently, particularly considering the growing global emphasis on natural products for health and wellness (Nasim *et al*., 2022). Among these plants, *Plectranthus vettiveroides*, a succulent aromatic herb endemic to southern India, has emerged as a noteworthy candidate for phytochemical exploration due to its rich traditional use in Ayurvedic and Siddha medicine. Initial research has shown that different extracts from its roots exhibit high biological activities, including antioxidant, antidiabetic, and anticancer properties (Lukhoba *et al*., 2006; Krubaa *et al*., 2024). This finding is consistent with the general scientific discourse highlighting the health-promoting activities of phytochemicals, especially those from plants, against chronic diseases, without the side effects commonly associated with synthetic drugs.

While these encouraging initial results are promising, there remains a significant shortfall in the holistic characterization of *Plectranthus vettiveroides* phytochemical profile and exhaustive assessment of its biological properties. Most of the current research has been centered mainly on individual extracts, without rigorous examination of the entire range of biologically active compounds contained within the roots of the plant. Furthermore, the mechanisms behind such biological activities remain poorly elucidated, restricting clinical application potential within treatment regimens (Athikkavil *et al*., 2023; Sailaja *et al*., 2021; Nisheeda *et al*., 2016). The traditional technique of soaking the roots of this plant in drinking water before consumption is the eye behind this study.

Moreover, the growing incidence of non-communicable diseases like diabetes, cancer, and cardiovascular disease requires the search for new therapeutic approaches from natural sources. The existing literature indicates that most plant-derived compounds possess multifaceted biological activities that may confer synergistic effects in therapeutic interventions (Samtiya*et al*., 2021; Tran *et al*., 2020). This makes research not only on individual compounds but also on the combined actions of intricate phytochemical mixtures present in whole plant extracts relevant.

Against these lacunae, this research is intended to systematically characterize the phytochemical constituents of the roots of *Plectranthus vettiveroides* and link the same to their corresponding biological activities. Through the use of sophisticated extraction and analytical methods, coupled with in vitro bioassays, the aim of this research is to add value to the therapeutic potential of *Plectranthus vettiveroides* (Sugavanam *et al*., 2014). Finally, this research aims to not only enhance the existing body of knowledge but also establish a foundation for future studies that could translate these findings into clinical practice, thereby addressing the urgent need for new and effective approaches to treating chronic diseases.

## Methodology

### *Collection and Preparation of Plant Extracts*

The roots of *Plectranthus vettiveroides* were procured from a traditional medical shop in Kumbakonam, Thanjavur District, Tamil Nadu. The roots of *Plectranthus vettiveroides* (Jacob) were collected from Baskar's old traditional plant products shop. The botanical identification and authentication were performed by Dr. N Ravichandran, CARISM SASTRA, Thanjavur. Taxonomic confirmation was performed using standard regional floras and herbarium comparison methods. After being dried, the roots were ground into a fine powder using a mixer grinder. To initiate the extraction process, 10 grams of the powdered *Plectranthus vettiveroides* root (vilamichai root) were macerated with 100 mL of ethanol for 24 hours, utilizing the intermittent shaking method. The resultant extract was filtered through Whatman filter No. 1 paper, with the filtrate reserved for subsequent phytochemical analysis. Dilutions of the extract in deionized water were prepared at concentrations of 100, 200, 300, 400, and 500 µg/mL for the in vitro studies. 10 grams of *Plectranthus vettiveroides* root powder were used for extraction. Extraction was performed with cold extraction using the maceration method into ethanol (100 ml) solvent for 24 hours. The extraction was performed using ethanol maceration, and the resulting filtrate was directly utilized for subsequent phytochemical and biological analyses. Since the crude extract was not concentrated to dryness and weighed following solvent removal, the extraction yield percentage could not be determined. The present study investigated the roots of Vila Michai rather than the more commonly studied roots of Vetti. The distinction between these two types of roots is clearly illustrated in Figures 1 and 2.

***Figure 1:*** *Root of Vetti*

***Figure 2:*** *Root of Vilamichai*

*Qualitative Preliminary Phytochemical Analysis*

Preliminary screening of the *Plectranthus vettiveroides* root extract for phytochemical constituents was conducted using standard methods. Sofowara (1982). Trease and Evans (1989); Harborne (1998). The analyses focused on identifying key phytochemicals such as tannins, saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthraquinones, polyphenols, coumarins, anthocyanins, emodins, and cardiac glycosides. For instance, the presence of tannins was assessed by boiling the extract with water, followed by the addition of ferric chloride, which indicated positive results through color changes. Similarly, various tests involving the addition of reagents such as dilute ammonia, acetic anhydride, and 10% sodium hydroxide enabled the identification of saponins, flavonoids, terpenoids, and other biologically significant compounds.

*Quantitative Analysis of Phytochemicals*

The total phenolic content of the root extract was determined spectrophotometrically using the Folin-Ciocalteu method, with gallic acid as a standard. The procedure involved mixing the extract with Folin-Ciocalteu's reagent and sodium carbonate, resulting in color development that was read at 750 nm. Similarly, total flavonoids and tannins were quantified using colorimetric methods involving specific reagents, and the results were expressed as equivalents of quercetin and tannic acid per gram of dried sample. Additionally, the total saponins and alkaloids were assessed following standardized protocols that involved colorimetric assessments and spectrophotometric measurements (Sharma *et al*., 2023).

*In Vitro Biological Activity Evaluations*

The bioactivity of *Plectranthus vettiveroides* extracts was assessed using various in vitro assays for antidiabetic, anti-inflammatory, anti-lipidemic, and immunomodulatory activities. The α-amylase and α-glucosidase inhibition activities of the extract were determined using varying concentrations of the extract with starch and paranitrophenyl-α-D-glucopyranoside substrates. In the anti-inflammatory assays, egg and bovine albumin denaturation inhibition was checked using diclofenac sodium as a control drug. Its capacity to stabilize membranes and reduce hemolysis again emphasized its anti-inflammatory potential.

*Antioxidant and Antiurolithiatic Activities*

Antioxidant activity was tested mainly through DPPH radical scavenging, hydroxyl radical scavenging, and nitric oxide scavenging assays. The extract proved to have remarkable free radical scavenging potential, reflecting great antioxidant potential. At the same time, nucleation and aggregation assays of calcium oxalate crystallization in antiurolithiatic activity were studied, where the extract efficiently prevented nucleation as well as crystal aggregation, ensuring its therapeutic use against urolithiasis (Hussen & Endalew, 2023; Habu & Ibeh, 2015; Raj *et al*., 2024).

Antiurolithiatic activity was evaluated using calcium oxalate nucleation and aggregation assays. The extract at various concentrations was incubated with calcium chloride and sodium oxalate solutions. Crystal formation and aggregation were assessed spectrophotometrically and microscopically. Percentage inhibition and $IC_{50}$ values were calculated by comparing extract-treated samples with control and standard drug-treated groups under identical conditions.

*Immunomodulatory Effects and Cytotoxic Activity*

Immunomodulatory activities were assessed with phagocytosis assays against *Candida albicans*. Fungal cells phagocytosed by neutrophils were quantified to calculate the phagocytic index, showing the capacity of the extract to stimulate immune responses. The nitro blue tetrazolium assay also supported the immunomodulatory effects by quantifying the oxidative burst activity of the neutrophils. In addition, cytotoxicity was determined against HeLa cells using the MTT assay, with findings indicating the extract's cytotoxicity toward cancer cells, highlighting its implication in cancer therapy (Miramón *et al*., 2012; Al-Oqai *et al*., 2021).

*Antibacterial Activity and Statistical Analysis*

The antibacterial activity of the *Plectranthus vettiveroides* extract against *Vibrio parahaemolyticus* and *Vibrio harveyi* was tested by the Müller-Hinton agar method (Uwizeyimana *et al*. 2020). Inhibition zones were noted for different concentrations, showing the potential of the extract as a natural antibacterial agent. Statistical analysis, such as triplicate tests and IC50 determination, was done using GraphPad Instat software (GraphPad Software Inc., USA), giving a complete understanding of the biological activities of the extract and supporting its potential in pharmacological applications.

In summary, the extensive phytochemical profiling and biological activity evaluations of *Plectranthus vettiveroides* root extract reveal its significant therapeutic potential. This highlights the need for further investigation into its active constituents and their mechanisms of action, which could lead to potential applications in health and medicine.

The extraction was performed using ethanol maceration, and the resulting filtrate was directly utilized for subsequent phytochemical and biological analyses. Since the crude extract was not concentrated to dryness and weighed following solvent removal, the extraction yield percentage could not be determined.

## Results

*Phytochemical Profile*

The observed biological activities may be attributed to the phenolic and flavonoid constituents identified in the extract. Phenolic compounds and flavonoids can inhibit α-amylase and α-glucosidase enzymes by interacting with their active sites, thereby reducing carbohydrate digestion and glucose release. In addition, flavonoids, terpenoids, and other polyphenols are known to induce apoptosis, generate oxidative stress in cancer cells, and interfere with cell-cycle progression. These mechanisms may collectively contribute to the antidiabetic and anticancer activities observed in the present study. The present study examined the phytochemical composition of *Plectranthus vettiveroides* root extracts using ethanol. Qualitative analysis revealed the presence of several bioactive compounds, including tannins, saponins, flavonoids, steroids, alkaloids, and anthraquinones, while terpenoids, triterpenoids, and glycosides were absent. Quantitative evaluations measured high concentrations of total phenols (223.57 mg GAE/g), flavonoids (173.19 mg QE/g), tannins (113.07 mg tannic acid equivalents/g), alkaloids (102.75 mg atropine equivalents/g), and saponins (88.21 mg Qu/g) from Figure 3.

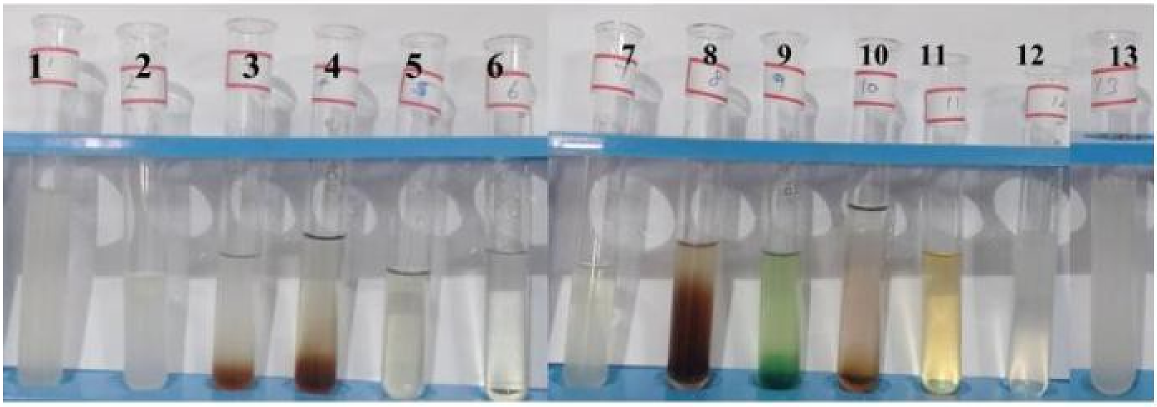



***Figure 3:*** *Phytochemicals Qualitative Analysis of Plectranthus vettiveroides Roots Ethanol Extract (1.Tannin, 2. aponin, 3. Flavonoids, 4. Steroids, 5. Terpenoids, 6. Triterpenoids, 7.Alkaloids, 8Anthroquinone, 9.Polyphenol, 10.Glycoside, 11.Coumarins, 12.Emodins,13.Anthocyanins)*

*Antidiabetic Activity*

The antidiabetic potential of *Plectranthus vettiveroides* was evaluated through its inhibitory effects on α-amylase and α-glucosidase enzymes. Inhibitory activity was dose-dependent, with maximum inhibition observed at 500 µg/ml for α-amylase (87.66%) and α-glucosidase (85.75%). The IC50 values were 275.88 µg/mL for the plant extract and 249.49 µg/mL for Acarbose, confirming the extract's significant antidiabetic properties (Table 1).

***Table 1:*** *In Vitro Antidiabetic Activity of Plectranthus vettiveroides Root Using Alpha Amylase Enzyme and Comparison with the Standard Drug Acarbose*

| Concentrations (µg/ml) | % of inhibitions | |
|---|---|---|
| | ***Plectranthus vettiveroides*** | **Acarbose (Standard)** |
| **100** | 19.76±1.07 | 21.88±1.15 |
| **200** | 34.74±0.98 | 40.45±1.13 |
| **300** | 57.29±1.31 | 60.07±0.98 |
| **400** | 71.35±1.29 | 78.64±1.09 |
| **500** | 87.66±1.11 | 96.15±1.24 |
| **$IC_{50}$ (µg/ml)** | 275.88 | 249.49 |

*Anti-inflammatory Activity*

Anti-inflammatory assays demonstrated the ethanolic extract's capacity to inhibit protein denaturation. The percentage of inhibition at 500 µg/mL was 88.22% for egg albumin denaturation, while for bovine serum albumin, it was 86.98%. The IC50 values for the extract were 272.99 µg/mL for egg albumin and 277.32 µg/mL for bovine serum albumin, indicating strong anti-inflammatory potential.

*Lipid-Lowering Activity*

Significant lipase inhibition was observed with the extract, showing a maximum reduction of 87.75% at 500 µg/mL, while cholesterol esterase inhibition reached 87.78%. The IC50 values were 290.32

µg/mL for lipase and 264.59 µg/mL for cholesterol esterase, highlighting the extract's potential as an anti-lipidemic agent.

*Antioxidant Activity*

The antioxidant capacity of the ethanol extract was assessed using various radical scavenging assays, including DPPH, nitric oxide, hydrogen peroxide, and iron chelation. The highest DPPH inhibition at 100 µg/mL was 86.14%, with an IC50 of 54.69 µg/mL. Other assays corroborated the extract's robust antioxidant activity, e.g., IC50 values of 57.95 µg/mL for nitric oxide and 54.59 µg/mL for hydrogen peroxide.

*Antiurolithiatic Activity*

In vitro studies demonstrated that the extract significantly inhibited the aggregation and nucleation of calcium oxalate (CaOx) crystals, indicating its potential antiurolithiatic activity. At a concentration of 500 µg/mL, the reduction in crystal aggregation reached 87.04%, with an $IC_{50}$ value of 281.86 µg/mL, showing an effect comparable to that of the standard drug, Cystone.Figure 5. Representative photomicrographs of calcium oxalate crystals observed under a light microscope at 40× magnification in the control and *Plectranthus vettiveroides* extract-treated groups using the aggregation assay.Figure 6. Representative photomicrographs of calcium oxalate crystals observed under a light microscope at 40× magnification in the control and *Plectranthus vettiveroides* extract-treated groups using the nucleation assay.

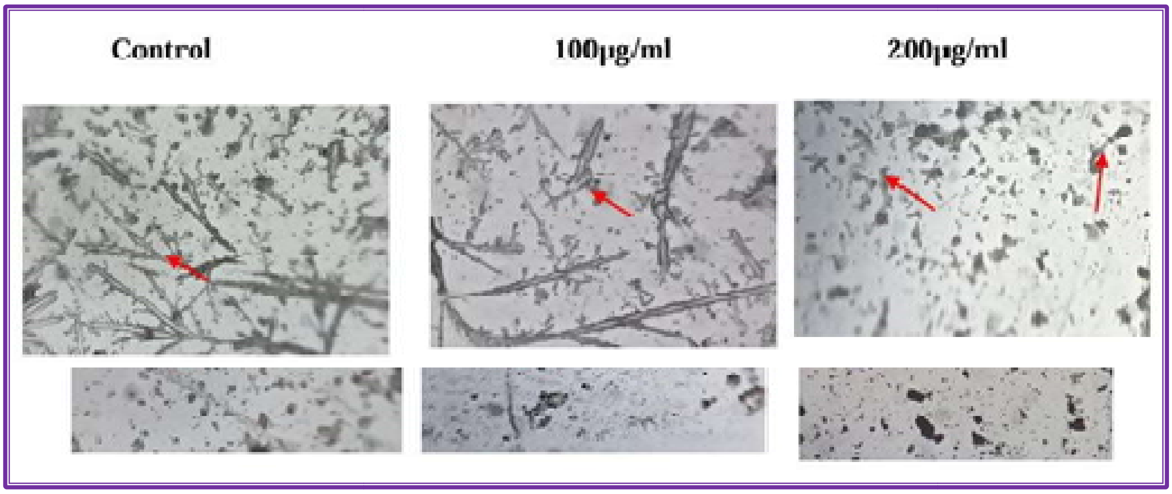



***Figure 4:*** *Representative Photographs of Caox Crystals as Observed Under a Light Microscope (40x) In Control and Plectranthus vettiveroides Extract Using the Aggregation Assay*

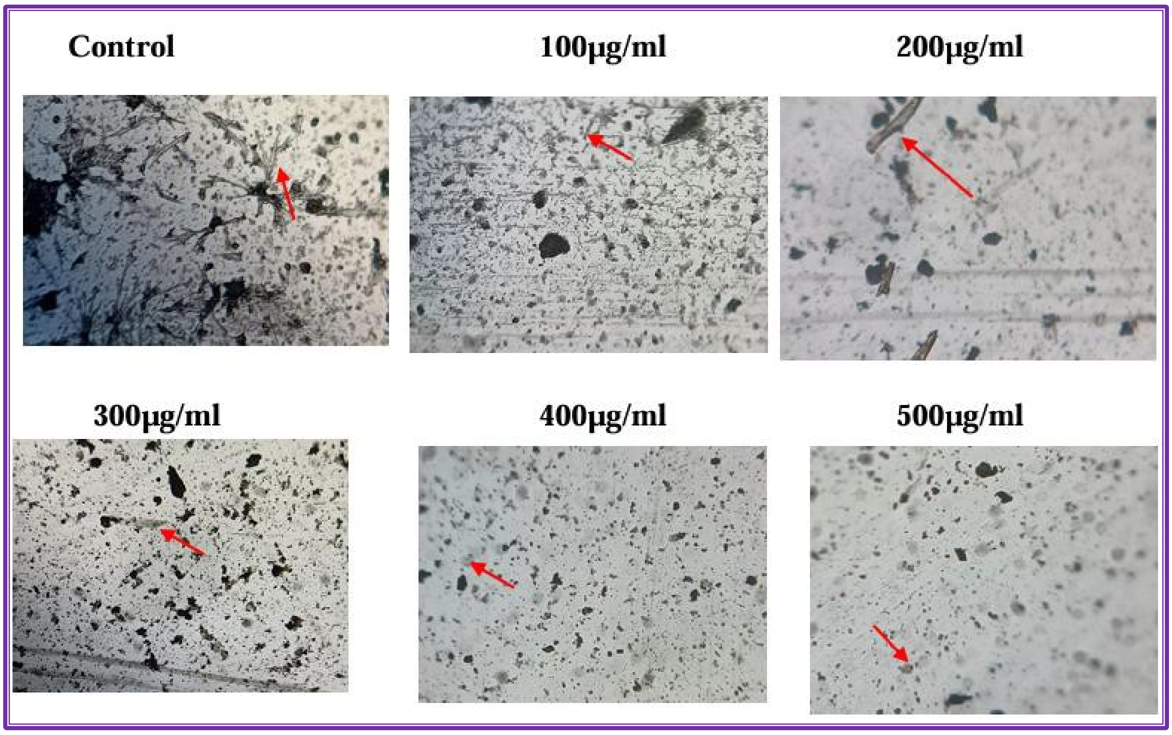



***Figure 5:*** *Representative Photographs of Caox Crystals as Observed Under a Light Microscope (40x) in Control and Plectranthus vettiveroides Extract Using the Nucleation Assay*

*Immunomodulatory Activity*

The extract enhanced macrophage phagocytic activity, producing a dose-dependent increase in the phagocytosis of *Candida* species. Phagocytic activity increased from 6.0% in the control group to 44.0% at 100 µg/mL, representing more than a sevenfold increase. These findings suggest that *Plectranthus vettiveroides* extract may enhance innate immune responses and phagocyte-mediated microbial killing.The nitroblue tetrazolium (NBT) assay showed a similar dose-dependent pattern, with a significant increase in the percentage of NBT-positive cells as the extract concentration increased. Because NBT reduction reflects activation of the respiratory burst pathway in phagocytic cells, these results indicate that the extract may improve innate immune cell function.

*Anticancer Activity*

Cytotoxicity studies on HeLa cervical cancer cells indicated that the extract exhibits promising anticancer properties with an IC50 of 267.44 µg/ml. Increasing concentrations resulted in noticeable morphological changes in the cells, underscoring the extract's capacity to induce apoptosis. Figure 6. Effect of varying concentrations of *Plectranthus vettiveroides* extract on cytotoxicity of HeLa (cervical cancer) cell line as determined by MTT assay (arrow mark indicates repre*sentative apoptotic cells).*

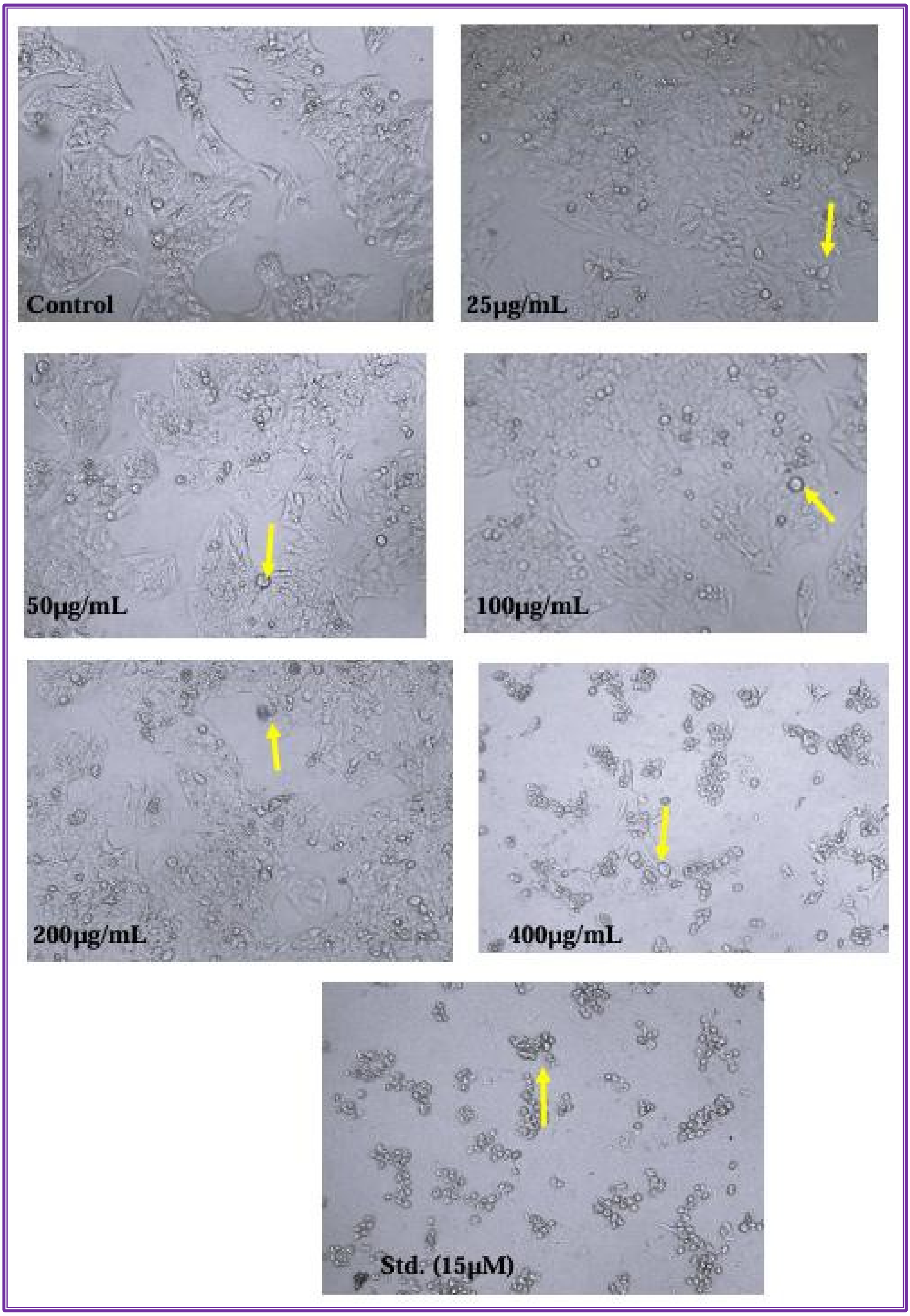
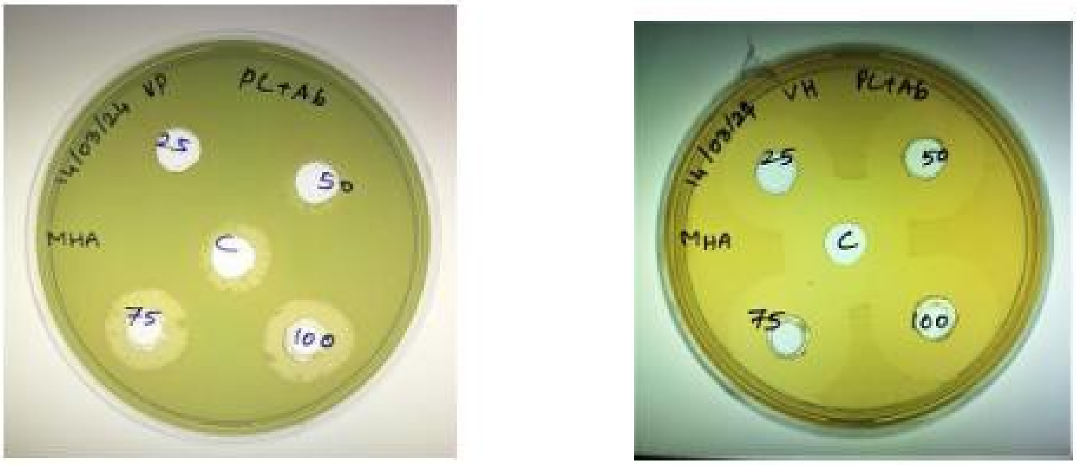



***Figure 6:*** *Effect of Varying Concentrations of Plectra thus Vettiveroides Extract on Cytotoxicity of Hela (Cervical Cancer) Cell Line as Determined by MTT Assay (Arrow Mark Indicates Representative Apoptotic Cells)*

*Antibacterial Activity*

Bacterial inocula were adjusted to approximately 1 × $10^8$ CFU/mL using the McFarland standard. Plates were incubated at 37°C for 24 h. Ciprofloxacin (10 µg/disc) served as the positive control, while solvent-treated discs served as negative controls. The antibacterial efficacy of the extract against *Vibrio parahaemolyticus* and *Vibrio harveyi* demonstrated significant growth inhibition, particularly on *Vibrio parahaemolyticus*, with the extract showcasing a dose-dependent antibacterial effect. Figure 7. MHA plate swabbed with *Vibrio parahaemolyticus.* C as a positive control (antibiotic) and 4 different concentrations of herbal extract. Table 2 represents the overall bioactivities of the extract with concentration, inhibitory percentage and IC50 values.




**Vibrio parahaemolyticus** **Vibrio harveyi**

***Figure 7:*** *MHA Plate Swabbed with Vibrio Parahaemolyticus. C As a Positive Control (Antibiotic) and 4 Different Concentrations of Herbal Extract*

***Table 2:*** *Consolidated Table for The Other Bioactivities of the Extract*

| Activity | Method / Measurement | Concentration (Mg/Ml) | Inhibition / Effect | Ic50 (Mg/Ml) |
|---|---|---|---|---|
| Antidiabetic Activity | α-Amylase Inhibition | 500 | 87.66% | 275.88 |
| | α-Glucosidase Inhibition | 500 | 85.75% | 274.84 |
| Anti-inflammatory Activity | Egg Albumin Denaturation Inhibition | 500 | 88.22% | 272.99 |
| | Bovine Serum Albumin Denaturation Inhibition | 500 | 86.98% | 277.32 |
| Lipid-Lowering Activity | Lipase Inhibition | 500 | 87.75% | 290.32 |
| | Cholesterol Esterase Inhibition | 500 | 87.78% | 264.59 |
| Antioxidant Activity | DPPH Scavenging | 100 | 86.14% | 54.69 |
| | Nitric Oxide Scavenging | - | - | 57.95 |
| | Hydrogen Peroxide Scavenging | - | - | 54.59 |
| Antiurolithiatic Activity | Calcium Oxalate Crystal Aggregation | 500 | 87.04% | 281.86 |
| Immunomodulatory Activity | Macrophage Phagocytosis Increase | 100 | 44.00% | - |
| Anticancer Activity | Cytotoxicity on HeLa Cells | - | - | 267.44 |
| Antibacterial Activity | Inhibition of Vibrio parahaemolyticus Growth | - | Significant Growth Inhibition | - |
| | Inhibition of Vibrio harveyi Growth | - | Dose-dependent effect | - |

All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). The immunomodulatory assays, including phagocytosis stimulation and Nitroblue Tetrazolium (NBT) reduction tests, were independently conducted in triplicate, and the average values were reported.

**Discussion**

The genus *Plectranthus*, particularly *Plectranthus vettiveroides*, has attracted considerable scientific interest because of its diverse bioactive constituents and broad therapeutic potential (Abdel-Mogib*et al*., 2002). The present study focused on the roots of *P. vettiveroides* and complements previous investigations of the genus that have examined its morphology, phytochemical composition, and biological activities. Comparison with earlier studies revealed both consistent and distinctive findings, thereby contributing to a broader understanding of the medicinal potential of this plant.The detailed phytochemical characterization conducted in this study is consistent with previous reports indicating that *Plectranthus* species contain numerous secondary metabolites, including flavonoids, phenolic compounds, and terpenes. In the present study, flavonoids and phenolic compounds were detected in substantial amounts in the roots of *P. vettiveroides*. These compounds may contribute to the reported antioxidant and anti-inflammatory activities of the extract. This finding agrees with the general consensus in the literature that species of the genus *Plectranthus* are rich sources of flavonoids and phenolic compounds.Previous studies have also investigated the antimicrobial properties of other *Plectranthus* species, including *P. scutellarioides*, and have identified several flavonoid constituents with notable antibacterial activity. Although the type and concentration of phytochemical constituents may vary among different plant parts, the roots of *P. vettiveroides* appear to make an important contribution to the plant's overall therapeutic potential.

In an intriguing study conducted by Nor and colleagues on P. scutellarioides, the plant exhibited effectiveness against multiple pathogens and a broad spectrum of bactericidal activity (Bismelah*et al*. 2022). In this study, it also demonstrated the antibacterial properties through the analysis of the extract of the root and observed a synergistic effect on microbial growth with P. vettiveroides roots. In addition, the data indicate that they have the capacity to induce apoptosis in cancer cells. This information is consistent with the observations regarding the bioactive fraction PVF obtained from the leaves, which presented a selective response to target cancer cells to induce apoptosis in colon cancer cells. The two extracts may share similar constituent fillers exhibiting pharmacological potential and indicate that leaf and root extracts are potential sources of anti-cancer agents, which aligns with the ongoing research of Plectranthus species. In addition to the study of anti-cancer potential, the anti-inflammatory properties also demonstrated that treatment with the extracts indicated a marked inhibition of inflammation, substantiating the evidence presented in the studies noted and providing support for the anti-inflammatory effects of other species within the genus Plectranthus. This evidence is relevant to the traditional use of the plants for treating a variety of ailments, including inflammation-related illnesses. The system in the regulation of the anti-inflammatory action, especially in terms of reducing the response from external factors, is critical to implementing basic principles not only of anti-inflammatory potential but also of the studies that chemical derivatives from *P. vetiveroides* could provide for more efficacious anti-inflammatory agents.

In addition, investigating the antihyperlipidemic effects of *P. vettiveroides* extends its potential use as a therapy and possesses important clinical significance for treating lifestyle diseases, including diabetes and cardiovascular disease. The reduction of serum cholesterol and triglycerides in dexamethasone-induced hyperlipidemia is consistent with other studies that have indicated that several plants within Lamiaceae can be utilized for lipid management. This benefit points to the potential innovative versatility of *P. vettiveroides* as a possibility for the formation of natural antihyperlipidemic preparations. (Kowalczyk *et al*., 2022); (Nallathambi *et al*., 2021) Additionally, the preparation of chitosan nanoparticles from the root extract enhances the applicability of *P. vettiveroides* as a drug delivery system, as evident in existing studies that establish the use of nanotechnology in the field of medicinal plants. In conjunction with an understanding of the previous work, the incorporation of *P. vettiveroides* chitosan nanoparticles can accomplish dual action (i.e., bioavailability and lipid management) using a dual-action natural biomaterial, leading to the use of the bioactive compounds of the plant that can have functional support in a medicinal application, not only its application as a drug delivery enhancement. Tamilarasu and Thamizhiniyan (2025) reported significant antioxidant activity in Plectranthus species attributed to their phenolic constituents, which supports the antioxidant findings of the present study. Sailaja *et al*. (2021) demonstrated anti-inflammatory properties of Plectranthus extracts through inhibition of inflammatory mediators, consistent with the observations. Nisheeda *et al*. (2016) identified flavonoid-rich fractions exhibiting free-radical scavenging activity, further supporting the role of polyphenols detected in the current investigation.

Saraswathy *et al*. (2011) reported the presence of essential oils and bioactive phytoconstituents in *Plectranthus vettiveroides* roots, suggesting their potential medicinal value. These findings support the present study, which demonstrated that the root extract contains multiple phytochemicals associated with diverse biological activities. Waldia *et al*. (2011) highlighted the antioxidant potential of medicinal plant extracts rich in phenolic compounds and flavonoids. Similarly, the present investigation identified appreciable levels of phenolics and flavonoids, which may contribute to the observed antioxidant activity of *Plectranthus vettiveroides* root extract.

Bismelah *et al*. (2022) demonstrated that plant-derived phytochemicals can exhibit significant anti-inflammatory and antimicrobial activities through their ability to modulate oxidative stress and inhibit microbial growth. The antibacterial and anti-inflammatory activities observed in the current study are consistent with these findings.

Herdiana *et al*. (2025) reported that phytochemical-rich plant extracts possess multiple biological activities, including antioxidant, immunomodulatory, and anticancer effects. In agreement with their observations, the present study demonstrated concentration-dependent immunomodulatory activity and cytotoxic effects against HeLa cancer cells, suggesting the potential contribution of bioactive secondary metabolites present in *Plectranthus vettiveroides* roots. The present study specifically investigated Vilamichaiver, that is, the roots of Vilamichai, rather than Vetti ver. The botanical identity of the plant material was independently confirmed by two experienced botanists.The strong antioxidant activity observed in the root extract may be attributed to its phenolic and flavonoid constituents. These compounds are known to donate hydrogen atoms or electrons to neutralize free radicals and interrupt oxidative chain reactions. Their ability to chelate transition metals further contributes to reducing oxidative stress.

The immunomodulatory activity of *Plectranthus vettiveroides* root extract was demonstrated by a concentration-dependent increase in both phagocytosis stimulation and Nitroblue Tetrazolium (NBT) reduction. In the phagocytosis assay, the percentage of killed Candida increased from 13.0% at 12.5 µg/mL to 44.0% at 100 µg/mL, approaching the activity of the standard pooled serum (48.0%). Similarly, NBT-positive cells increased from 19.0% to 63.0% across the tested concentrations, compared with 75.0% for the standard lipopolysaccharide-treated group. These findings suggest stimulation of innate immune responses through enhanced neutrophil phagocytic activity and respiratory burst mechanisms.

The observed immunostimulatory activity may be attributed to the presence of phenolic compounds, flavonoids, saponins, and terpenoids identified during phytochemical screening. These phytoconstituents have been reported to enhance macrophage and neutrophil functions, stimulate cytokine production, improve microbial clearance, and increase intracellular oxidative burst activity. The increased NBT reduction observed in the present study indicates enhanced generation of reactive oxygen species by activated phagocytes, an important mechanism involved in host defence against invading pathogens. Recent investigations further highlight the expanding scientific interest in the genus Plectranthus beyond its traditional medicinal applications. For example, Jose *et al*. (2026) developed fluorescent nanocellulose derived from *Plectranthus barbatus* for the selective detection of Pb (II) ions in aqueous systems, demonstrating the potential of Plectranthus species as valuable sources of functional biomaterials. Such studies, together with the present findings on the biological activities of *Plectranthus vettiveroides*, emphasize the broad pharmaceutical, biomedical, and biotechnological relevance of this genus and support further exploration of its bioactive constituents.

*Limitations*

The present study has several limitations that should be acknowledged. First, the investigation relied exclusively on in vitro assays, which may not fully reflect the complex biological environments of living organisms. Therefore, the observed biological activities cannot be directly extrapolated to clinical settings. Second, the extraction procedures employed may not have captured all phytochemicals present in the roots of *Plectranthus vettiveroides*, potentially overlooking compounds with relevant biological activity. Third, the study primarily focused on selected bioactive constituents, and possible synergistic interactions among the diverse phytochemicals present in the extract were not evaluated. Furthermore, toxicity studies, animal experiments, and clinical investigations were not performed. Consequently, the bioavailability, pharmacokinetic profile, long-term safety, and therapeutic efficacy of the identified phytoconstituents remain unknown. The findings should therefore be interpreted as preliminary evidence of biological potential rather than definitive proof of therapeutic applicability.

*Future Scope*

Future research should focus on bioassay-guided fractionation and isolation of the active phytoconstituents responsible for the observed biological activities. Detailed investigations into the molecular mechanisms underlying these effects are also warranted. In addition, comprehensive toxicity assessments, pharmacokinetic studies, and bioavailability evaluations should be conducted to establish the safety profile of the extract and its constituents. Validation of the present findings in appropriate animal models will be essential to confirm in vivo efficacy and therapeutic potential. Furthermore, studies exploring synergistic interactions among phytochemicals may provide a more comprehensive understanding of the extract's biological effects. Ultimately, well-designed clinical studies will be required to determine the safety, efficacy, and therapeutic applicability of *Plectranthus vettiveroides* root extract in humans.

## Conclusion

The examination of the root extract of *Plectranthus vettiveroides* showed significant in vitro therapeutic effects through comprehensive phytochemical characterization and biological studies. Different bioactive compounds, such as tannins, flavonoids, and phenolics, are all present in the extract, which exhibits strong antioxidant, anti-diabetic, anti-inflammatory, and anti-cancer activity. The extract shows a strong inhibition of major enzymes involved in diabetes, significant anti-inflammatory activity, and considerable cytotoxic effects against HeLa cancer cells. Furthermore, the antihyperlipidemic effect shows its relevance for cardiovascular disease management. The present study demonstrates that *Plectranthus vettiveroides* root extract possesses promising immunomodulatory activity under in vitro conditions. However, further investigations involving animal models, toxicity evaluation, and mechanistic studies are required before its therapeutic potential can be established. This study is unique because it specifically examines the roots of the plant in relation to their use, the roots of the plant in the context of its usage in drinking water. The findings demonstrate that *Plectranthus vettiveroides* root extract possesses promising in vitro biological activities. However, further *in vivo*, toxicological, and clinical studies are necessary before therapeutic applications can be established.

## Conflict of the Interest

There is no conflict of interest: There is no external financial aid.

**Acknowledgement**

All the authors have significantly contributed to the research and drafting of the manuscript. Authors sincere acknowledgements to P.S Yogitha PhD scholar, SASTRA(SRC) for her help in the manuscript preparation.

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