Research Article

Exploration of Bioactive Constituents and Evaluation of Antimicrobial and Anti-Inflammatory Activities of Ethanolic Leaf Extract of *Paederia foetida* via GC–MS Analysis

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ABSTRACT

The phytochemical profile and biological activities of the ethanolic leaf extract of *Paederia foetida* are examined in this work, with a focus on the antibacterial and anti-inflammatory potential. A wide variety of bioactive components, such as phenolic compounds, fatty acids, terpenoids, and hydrocarbons, which are known to contribute to different pharmacological effects, were found by a Gas Chromatography–Mass Spectrometry (GC–MS) study. Ciprofloxacin was used as the standard reference to assess antibacterial activity against soil-derived microbial strains using the agar well diffusion method. With a distinct concentration-dependent rise in zones of inhibition and noticeable activity at higher doses, the extract showed strong antibacterial efficacy. Additionally, the extract demonstrated a significant suppression of inflammatory responses in in vitro models used to evaluate the anti-inflammatory effects. The observed bioactivities could be caused by the presence of important phytoconstituents such as 2,4-di-tert-butylphenol and Neophytadiene. Overall, the results support the plant's traditional medical use and point to its potential as a natural source of powerful antibacterial and anti-inflammatory therapeutic compounds.

Keywords: *Paederia foetida*, GC–MS analysis, antimicrobial activity, Ethanolic leaf extract.

INTRODUCTION

It has long been known that medicinal plants are important sources of bioactive substances with a wide range of therapeutic uses. Due to their potential safety, cost-effectiveness, and efficacy in comparison to synthetic medications, plant-derived natural compounds have drawn more attention in recent years, especially in the treatment of inflammatory and microbial illnesses[1]. The search for novel bioactive agents from plant sources has become more intense due to the increasing prevalence of antimicrobial resistance among pathogenic microorganisms[2]. *Paederia foetida* is a perennial climbing plant that is widely distributed throughout tropical and subtropical regions of Asia and is a member of the Rubiaceae family. It has long been used in traditional medicine to treat a variety of gastrointestinal conditions, inflammation, diarrhea, and microbial infections[3]. According to earlier research, *P. foetida* has a wide spectrum of pharmacological effects since it is rich in phytoconstituents like alkaloids, flavonoids, terpenoids, and phenolic compounds[4]. A potent analytical method that is widely used to identify volatile and semi-volatile chemicals found in plant extracts is gas chromatography–mass spectrometry (GC–MS). It facilitates the relationship between phytoconstituents and their biological actions and offers comprehensive insights into chemical composition[5].

Thus, the current work intends to investigate the phytochemical profile of the ethanolic leaf extract of *P. foetida* using GC–MS analysis, as well as to assess its antibacterial activity against microbial strains taken from soil and its potential for in vitro anti-inflammatory effects. In addition to highlighting the plant's potential as a natural source of potent antibacterial and anti-inflammatory compounds, the study attempts to substantiate the plant's traditional therapeutic claims.

MATERIALS AND METHODS

Plant Collection and Extraction:

Fresh leaves of *Paederia foetida* were gathered from their natural habitat. To create a consistent coarse substance, the leaves were cleaned, shade-dried, and ground into a powder. The plant's powder was gathered, and it was macerated with ethanol for 73 hours at room temperature with sporadic stirring. Man No. 1 filter paper was used after the extracts were filtered through multiple layers of muslin cloth for coarse filtration to remove residues. The same process was used to further remove the residue. After combining the filtrates, they were dried by evaporation at a temperature of no more than 35 °C. Before being used, the extracts were stored in a cool location.

GC–MS Analysis:

Nowadays, GC-MS is widely recognized as an essential technological platform for secondary metabolite profiling in plant-based materials. Here, different substances within a test sample can be separated based on how long they remain by utilizing the features of both gas chromatography and mass spectrometry. The active principles in this study are shown in Figures 1 and Table 1, which show that the ethanolic extract of *Paederia foetida* contains a number of bioactive phytochemical compounds. These compounds include retention time (RT), molecular formula, molecular weight (MW), concentration (peak area%), chemical structures, and biological activities.[6]

GC-MS, which only requires a small sample of plant extract, is one of the best, fastest, and most dependable methods

for identifying various phytochemicals, including polyphenolic compounds, alkaloids, long-chain hydrocarbons, and unsaturated fatty acids.[7] Consequently, the GC-MS approach was used in the current investigation to locate and identify the phytochemical components of the selected medicinal plant.

The Shimadzu GC-MS-QP2010 Ultra Version of the device was used to perform GC-MS analysis of the ethanolic extract of *Paederia foetida*. This equipment can also be used for precise two-dimensional GC and ultra-high separation. Using a capillary standard non-polar fused silica column, helium is used as the mobile phase carrier gas in this system with a total flow rate of 14.1 ml/min, column flow of 1 ml/min, and purge flow of 3.0 ml/min. The injector was running at 280 °C, and the column oven was set at 70 °C. The split-less injection approach was used with the AOC-20i auto injector at a pressure of 61.3 kPa (psi). The gadget was fully operational to detect compounds with a mass per charge (m/z) ratio between 40 and 850 atomic mass units (amu) when the 1 µl sample ethanolic extract was inserted.

The GC-MS parameters also include a solvent cut time of 4.5 minutes, an elution duration of 63 minutes, an interface temperature of 260 °C, and an ion source temperature of 200 °C. The NIST 20 (2020) mass spectrum library search tool was used to compare the data. Retention times ranging from five to sixty-three minutes were noted for the study of twenty-five compounds, along with each chemical's percentage area, molecular formula, and molecular mass.

Retention time (RT), molecular formula, molecular weight (MW), concentration (%), and previously reported biological activity by other studies are among the key criteria for examination for specific compounds that are displayed in Table 1, along with the GC-MS chromatogram depicted in Figure 1. In the current work, these methods help predict the formula and structure of a number of biomolecules.

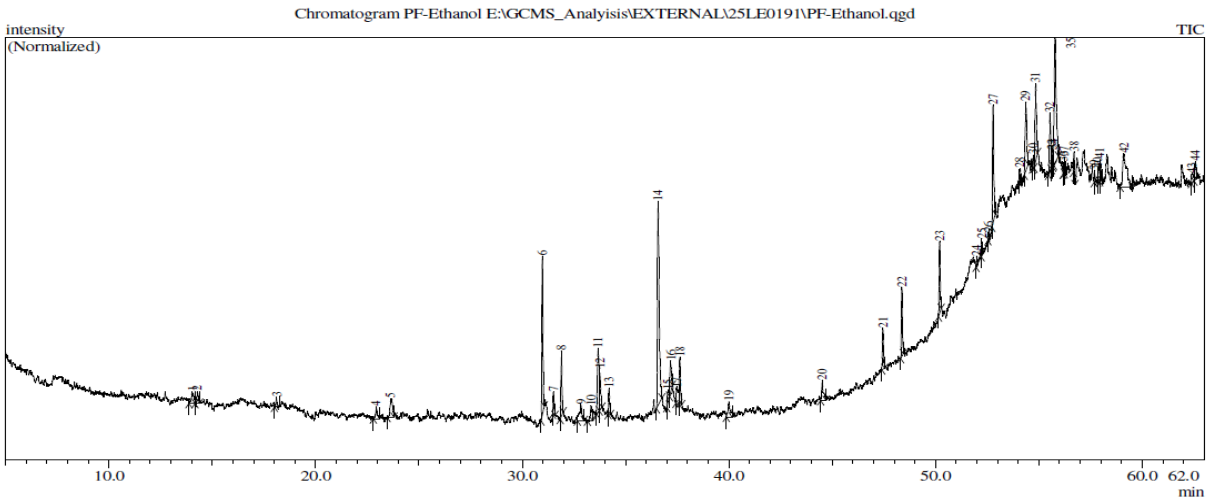
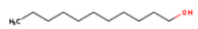
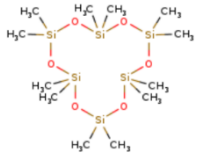
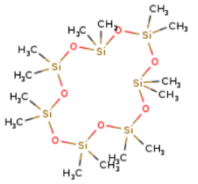
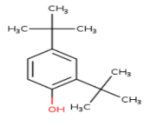
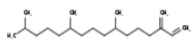
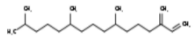
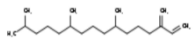
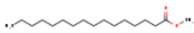
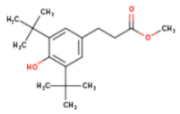
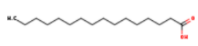
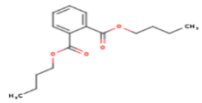
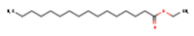
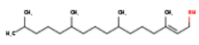
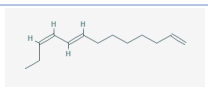
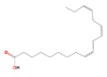
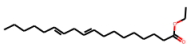
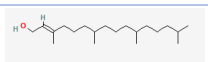
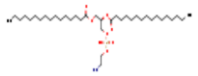
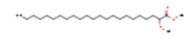
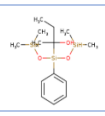
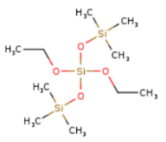


Figure 1: GC-MS chromatogram for ethanolic extract of *Paederia foetida*

Table 1: GC-MS spectral analysis of ethanolic extract of *Paederia foetida*

Peak no	RT	Compound name	Area %	Mol. formula	Mol. Wt(g/mol)	Reported bioactivity	Structure	Ref
1	14.028	1-Undecanol	1.33	$C_{11}H_{24}O$	172	antimicrobial and antifungal		***8***
2	14.285	Undecane	0.67	$C_{11}H_{24}$	156	antimicrobial		***9***
3	18.106	Cyclohexasiloxane, dodecamethyl-	0.32	$C_{12}H_{36}O_6Si_6$	444	antimicrobial and antioxidant activities.		***10***
4	22.942	Cycloheptasiloxane, tetradecamethyl-	0.92	$C_{14}H_{42}O_7Si_7$	518	antimicrobial		***11***

5	23.645	2,4-Di-tert-butylphenol	2.03	$C_{14}H_{22}O$	206	antibacterial, antifungal,		***12***
6	30.978	Neophytadiene	7.35	$C_{20}H_{38}$	278	anti-inflammatory		***13***
7	31.513	Neophytadiene	0.94	$C_{20}H_{38}$	278	anti-inflammatory		***14***
8	31.901	Neophytadiene	2.80	$C_{20}H_{38}$	278	anti-inflammatory		***15***
9	32.838	Hexadecanoic acid, methyl ester	1.66	$C_{17}H_{34}O_2$	270	antimicrobial, antioxidant		***16***
10	33.332	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	1.15	$C_{18}H_{28}O_3$	292	antimicrobial activity.		***17***
11	33.676	n-Hexadecanoic acid	4.09	$C_{16}H_{32}O_2$	256	antimicrobial, antioxidant		***18***
12	33.758	Dibutyl phthalate	2.28	$C_{16}H_{22}O_4$	278	antibacterial and antifungal		***19***
13	34.206	Hexadecanoic acid, ethyl ester	0.98	$C_{18}H_{36}O_2$	284	antimicrobial and antioxidant		***20***
14	36.578	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	13.72	$C_{20}H_{40}O$	296	antioxidant, anti-inflammatory		***21***
15	37.042	1,E-8,Z-10-Tridecatriene	0.92	$C_{13}H_{22}$	178	antimicrobial and insecticidal properties		***22***
16	37.181	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	1.83	C18H30O2	278	anti-inflammatory, antioxidant		***23***
17	37.475	9,12-Octadecadienoic acid, ethyl ester	1.39	$C_{20}H_{36}O_2$	308	anti-inflammatory		***24***
18	37.626	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	2.21	$C_{18}H_{30}O_2$	278	anti-inflammatory, antioxidant		***25***
19	40.007	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.45	$C_{20}H_{40}O$	296	Antioxidant activity		***26***
20	44.518	Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl]oxy]methyl]-1,2-ethanediyl ester	1.54	$C_{37}H_{74}NO_8P$	691	membrane-protective and antimicrobial activities.		***27***
21	47.452	Docosanoic acid, docosyl ester	1.96	$C_{44}H_{88}O_2$	648	antioxidant and antimicrobial		***28***
22	48.373	Squalene	2.87	$C_{30}H_{50}$	410	antioxidant, anticancer		***29***
23	50.204	Tricosanoic acid, 2-methoxy-, methyl ester	3.08	$C_{25}H_{50}O_3$	398	antimicrobial and antioxidant activities.		***30***
24	51.983	Ethoxy(phenyl)silanediol, 2TMS	0.73	$C_{14}H_{28}O_3Si_3$	328	antimicrobial and bioactive properties.		***31***
25	52.238	Silicic acid, diethyl bis(trimethylsilyl) ester	0.47	$C_{10}H_{28}O_4Si_3$	296	antioxidant and antimicrobial		***31***

EVALUATION OF ANTIMICROBIAL ACTIVITY:

The antibacterial activity of the ethanolic leaf extract of *Paederia foetida* was evaluated using standard microbiological techniques and the agar well diffusion method. In this study, soil-derived microbes were used as test organisms to assess the extract's broad-spectrum antibacterial capabilities. Following the collection of aseptic soil samples, microbial cultures were isolated and maintained on nutrient agar media. The inoculum was made of freshly created soil microorganisms floating in a sterile saline solution. The turbidity was adjusted to meet the 0.5 McFarland standard, or approximately 1×10^2 CFU/mL, in order to preserve similar microbial density.

Sterile feeding agar plates were prepared and allowed to harden under aseptic conditions. Using a sterile cotton swab, the standardized microbial suspension was uniformly spread throughout the agar plate surface to produce a confluent lawn of growth. Wells around 6 mm in diameter were aseptically bored into the agar using a sterile cork borer, being careful to provide enough space between wells to avoid inhibitory zone overlap.

Different quantities of ethanolic extract were carefully applied to the wells using a micropipette. Ciprofloxacin was used as the conventional antibiotic (positive control) to compare the plant extract's antibacterial efficacy, and ethanol was used as the negative control to validate the lack of solvent-induced activity. To encourage microbial growth and extract diffusion into the surrounding media, the plates were next incubated for 24 hours at 37°C. After incubation, the plates were examined for clear zones of inhibition surrounding the wells. The diameter of the zones was measured in millimeters using a calibrated scale. Each experiment was conducted in triplicate, and the findings were expressed using the mean ± standard deviation. A concentration-dependent increase in the zone of inhibition showed that the extract had significant antibacterial action against microorganisms obtained from soil.

Zone of inhibition with standard deviation:

Concentration	Standard	PFME	PFEE	PFCE	PFEAE
(µg/ml)	(ZOI in mm)	**(**ZOI in mm)	(ZOI in mm)	(ZOI in mm)	(ZOI in mm)
100	**15**	**13**	**12**	**11**	**10**
150	**18**	**15**	**14**	**13**	**12**
200	**22**	**18**	**17**	**16**	**15**
250	**27**	**22**	**21**	**19**	**19**

Graphical Interpretation of Results:

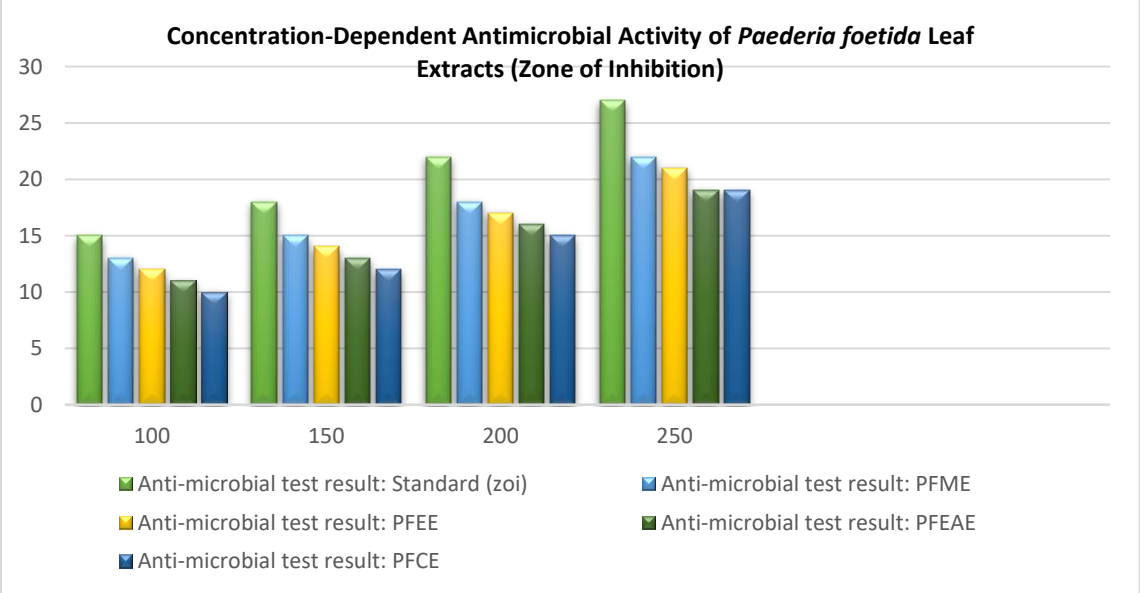


Figure 2: Standard curve

Interpretation of Results:

The graphical data from the antimicrobial experiment show that all of the tested extracts clearly increased the zone of inhibition against the chosen bacteria in a concentration-dependent manner. Due to variations in the distribution of bioactive components among the solvent fractions, **PFME** showed the strongest antibacterial activity among the extracts, followed by **PFEE, PFEAE**, and **PFCE.** The plant extracts had significant antibacterial activity, especially at higher doses (200–250 µg/ml), while the standard medication (ciprofloxacin) showed relatively stronger inhibitory effects at all dosages. These findings imply that

the extracts may be a promising natural source for the creation of substitute antibacterial agents and may contain bioactive components.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY:

The purpose of the in vitro anti-inflammatory study of *Paederia foetida* leaf extract is to offer evidence in favor of its traditional use in the management of inflammation. In response to injury, disease, or harmful stimuli, the body uses inflammation as a natural defense mechanism. However, continuous or severe inflammation can cause a number of health issues, such as autoimmune diseases, cardiovascular diseases, and arthritis. *Paederia foetida* leaf extract's in vitro anti-inflammatory effectiveness can be evaluated using the egg albumin denaturation assay, a popular method based on the theory that many anti-inflammatory medications lessen heat-induced protein denaturation.

Phosphate-buffered saline (pH 6.4) and various plant extract quantities (e.g., 100, 150, 200, and 250 µg/mL) are mixed with a known amount of fresh egg albumin in this experiment.

For comparison, a control sample (without extract) and a standard solution containing a known anti-inflammatory drug (such as aspirin or diclofenac sodium) are also prepared. After being incubated at 37°C for fifteen minutes, all mixes are heated to 70°C for five minutes to induce denaturation. After each sample has cooled to room temperature, its absorbance at 600 nm is measured using a UV-Vis spectrophotometer. The percentage inhibition of protein denaturation is then calculated using the following formula:

$$\% \text{ Inhibition} = [(A.\ C - A.\ T)/A.\ C] \times 100.$$

Here, A, C & T accordingly denote Absorption, Control & Test

A larger percentage of inhibition indicates stronger anti-inflammatory potential. This simple, repeatable method provides valuable preliminary data for further pharmacological study and can be used to accurately assess the anti-inflammatory activity of *Paederia foetida* leaf extract. In the case of *Paederia foetida* leaf extract, a significant proportion of inhibition demonstrates its promise as a natural anti-inflammatory medication and supports its traditional use in the treatment of inflammation-related disorders. Additionally, these findings offer helpful baseline data for further pharmacological evaluation via more complex in vivo and clinical studies. Such investigations can shed light on the extract's specific modes of action, therapeutic dosage, bioavailability, and safety profile.

Observation Table:

Concentration (µg/ml)	% Inhibition of Diclofenac sodium	% Inhibition of PFME	% Inhibition of PFEE	% Inhibition of PFEAE	% Inhibition of PFCE
100	94.65	40.62	42.40	44.32	21.64
150	96.52	55.78	58.32	48.14	30.54
200	98.37	63.34	60.21	51.87	35.34
250	99.24	68.68	63.45	58.18	42.97

Graphical Interpretation of Results:

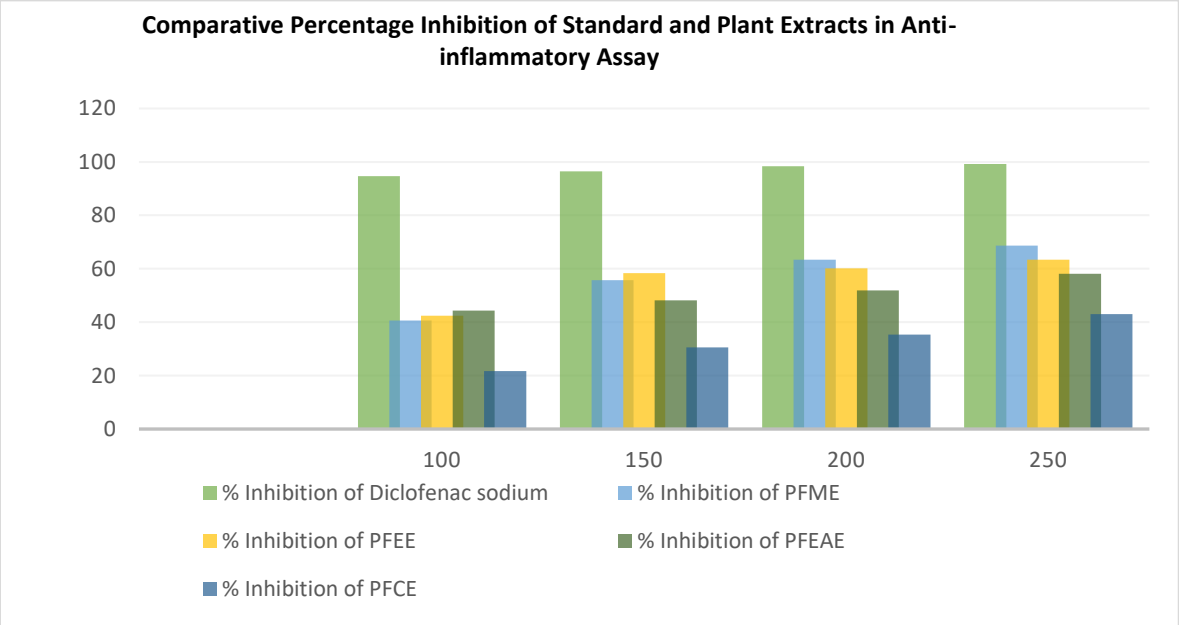


Figure 3: Standard curve

Interpretation of Results:

All extracts showed concentration-dependent inhibitory efficacy in the in vitro anti-inflammatory experiment. With 68.68% inhibition at 250 µg/ml, PFME had the strongest anti-inflammatory potential among the studied extracts, followed by PFEE (63.45%), PFEAE (58.18%), and PFCE (42.97%). The considerable action of PFME and PFEE indicates the presence of important bioactive phytoconstituents with anti-inflammatory effects, even if standard diclofenac sodium showed higher inhibition (99.24%). These results suggest that the extracts have promising anti-inflammatory activity and could be used as natural medicinal agents for conditions associated with inflammation.

CONCLUSION

All of the extracts showed dose-dependent antibacterial and anti-inflammatory properties, according to the study. Diclofenac sodium demonstrated the best activity as the standard in the anti-inflammatory experiment, while PFME and PFEE had the most inhibitory effects among the extracts. Similar to the standard medicine at higher dosages, PFME showed the largest zone of inhibition in the antibacterial investigation. The overall results indicate that the plant extracts, especially PFME and PFEE, include important bioactive components that have both antibacterial and anti-inflammatory qualities, suggesting their potential therapeutic utility in the treatment of microbial infections and inflammation.

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