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Research article

INFLUENCE OF EXTRACTION SOLVENTS ON THE ANTIBACTERIAL PROPERTIES OF *PAEDERIA FOETIDA* LEAF EXTRACTS AGAINST *E. COLI*

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Abstract

This study aimed to solvent selection for testing the medicinal plant Paederia foetida leaf extracts for combating bacterial infection. UV–VIS spectroscopy, therefore the extraction factor (EF) analysis and FTIR spectra indicate that ethanol is more efficient solvent over the acetone. Antibacterial activity against Escherichia coli was evaluated, and a concentration-dependent inhibition was observed. The antibacterial activity of the Paederia foetida leaf extracts consist of specific phytochemicals that may disrupt bacterial membrane integrity and inhibit metabolic pathways. Therefore, our findings illustrate Paederia foetida has the potential to be a promising resource for natural antibacterial agents, where ethanol is the preferred extraction solvent.

1. Introduction

Antibiotics are widely used to treat microbial infections; however, the emergence of antibiotic resistance has become a major concern. To address this issue, several alternative treatment strategies are being explored, including medicinal plants, which have long been used in traditional medicine for combating microbial infections. *Paederia foetida*, commonly known as skunk vine, is a perennial climbing vine belonging to the Rubiaceae family. It is indigenous to tropical and temperate regions of Asia, particularly in countries such as China, India, Japan, and Indonesia, and is also found in the Mascarenes, Melanesia, Polynesia, the Hawaiian Islands, and some southeastern parts of the United States (Langeland KA et al., 2008). In India, it is predominantly found in the north-eastern states. According to the eFlora of India, *Paederia foetida* is a habitant of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and West Bengal. It plays a significant role in traditional medicine for gastrointestinal ailments as well as local cuisine. Traditionally,

the plant has been used to treat a variety of ailments, including toothaches, dysentery, sores, enterosis, enteromegaly, rhinitis, rheumatism, edema, night blindness, and various digestive disorders such as gastritis, diarrhea, and ulcers (De S et al., 1994). Several studies have reported that extracts of *Paederia foetida* possess anti-inflammatory, antidiarrheal, antioxidant, antihepatotoxic, antitussive, and gastroprotective properties. The leaves are consumed either raw or boiled to relieve stomach aches. These regional uses highlight the ethnobotanical significance of *Paederia foetida* (De S et al., 1994; Afroz S et al., 2006; Osman H et al., 2009; De S et al., 1993; Nosaovaa G et al., 2007; Chanda S et al., 2015).

Such medicinal properties are due to the bioactive phytochemicals of *Paederia foetida*. These include iridoid glycosides such as asperuloside, scandoside, and paederoside; volatile oils like linalool, geraniol, and α-terpineol; triterpenoids including ursolic acid and oleanolic acid; as well as β-sitosterol, arachidic acid, alkaloids (paederine A and B), flavonoids, and significant amounts of essential minerals (Ojha S et al., 2018).

It has been reported that the leaf extracts of *Paederia foetida*, showed inhibitory effects against pathogenic bacteria (Yunita M, 2023). However, the mode of extraction plays a crucial role in determining the antibacterial efficacy, as it can influence the quantity and availability of secondary metabolites in the plant extract (Upadhyaya S, 2013). For example, ethanolic leaf extracts showed stronger antimicrobial activity than crude extracts. These active fractions also exhibited antibiofilm properties, effectively inhibiting biofilm formation and eradicating established biofilms (Priyanto JA et al., 2022).

In the present study, we investigate the antibacterial activity of *Paederia foetida* leaf extracts, which were obtained using two different solvents, ethanol and acetone, against gram-negative bacteria.

2. Materials and methods

2.1. Materials

Spectroscopic-grade Ethanol and Acetone were purchased from Spectrochem (India). All other chemicals/reagents were from Sigma-Aldrich, USA; otherwise specified in the corresponding section of the manuscript. Dried powder of *Paederia foetida* leaves was used for the downstream studies.

2.2. Methods

2.2.1. Preparation of ethanolic and acetone leaves extract of Paederia foetida

First, the leaves (*Paederia foetida*) were dried in the subjected to a rotary evaporator for several hours. The extract samples obtained from the rotary evaporator were dried. Stock solution (100 mg/ml) in DMSO for each solvent extract was prepared and followed preparation of a 1 mg/ml sub-stock solution. The whole process is represented in Figure 1.

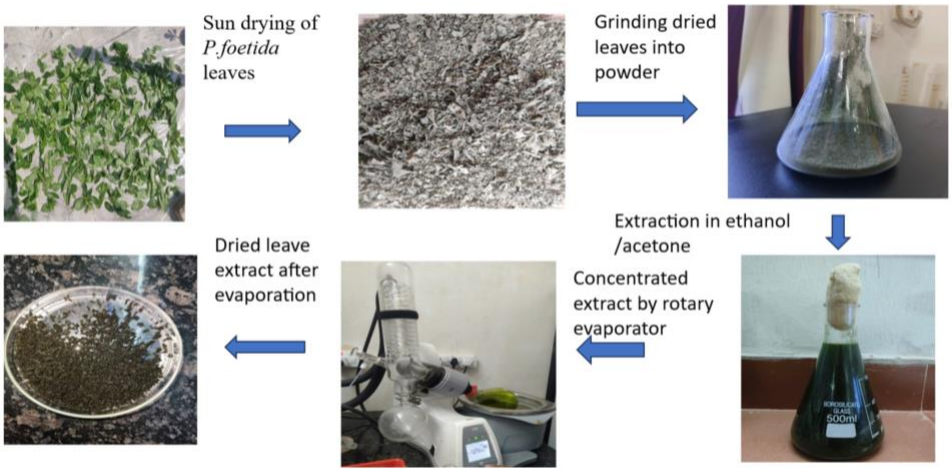

Figure 1. The complete flow for the preparation of ethanol and acetone extract from *Paederia foetida* leaves.

2.2.2. UV-Vis studies:

The steady-state absorption spectra were recorded in a UH–5300 (Hitachi, Tokyo, Japan) spectrophotometer, equipped with a pulsed Xe-lamp. In the spectrophotometer, the slit width was maintained at 2.5 nm, and the entire data was recorded within the range of 250nm-700nm. Spectroscopic-grade Ethanol and Acetone were purchased from Spectrochem (India) and used for making the experimental solutions. To achieve a homogeneous solution of the plant extracts, the mixture was stirred using a magnetic stirrer and kept undisturbed for ~5 min, and finally proceeded for the spectroscopic measurements.

2.2.3. FTIR study of both plant extracts:

The Fourier Transform Infrared spectrum (FTIR) of each extract was recorded in the IR region, from 4000 to 500 cm^{-1}, using a BRUKER FTIR spectrometer. The spectra were registered as evaporated extracts.

2.2.4.Antibacterial activity of both leaf extracts on agar plate by the well diffusion method

To study the antibacterial effect of both ethanolic and acetone leaves extract of the *Paederia foetida,* 1.5% of nutrient agar and 1.3% of nutrient broth were used for the preparation of agar plates. We used the well diffusion method for the antibacterial activity experiment. For that, 200 µl of *E. coli* culture was spread on the agar plates and incubated the plates for 30 minutes at 37°C in an incubator. Well was made by using 200 µl tips, and the agar plates were labelled as NIL, DMSO, 10, 30, 50, 70, 80, 100 µg/ml for both ethanol and acetone plates aseptically. The plant extract of six different concentrations was loaded in each well, except for the control (NIL), and kept in an incubator for 24 hours. We kept blanks for control, and we added 100µl of DMSO in one of the wells to check its effect. After 24 hours, the zone of inhibition was measured and recorded.

2.2.5. Effect of both leaf extracts on the growth of E. coli bacteria in broth media

In that connection, bacterial nutrient broth culture was prepared, and *E. coli* bacteria were treated with different concentrations of both ethanol and acetone extracts (0-100µg/ml) and kept in an incubator shaker at 37°C for overnight. The OD values of bacterial culture were measured at 600nm. Based on the OD value, we plotted the graph of OD against concentrations of plant extract.

3. Results and discussions

3.1. UV–VIS spectra

The UV–VIS spectra (700–250 nm) were recorded for *Paederia foetida* extract (in acetone [red] and ethanol [cyan] solvents), and the wavelengths of specific absorption maxima of poly-phenolic compounds (330 nm), carotenoids and terpenoids (400–450nm), and/or chlorophylls (665 nm) were identified (Fig. 2) (Dutta PP et al., 2023; Ojha S et al., 2018; Caunii A et al., 2012). To compare the yields of extraction in two solvents, the Extraction Factors (EF) of the bioactive molecules from each extract have been calculated considering the absorbance ($A_{\lambda max}$) recorded for each corresponding λ_{max} value, multiplied by the dilution factor (d) (Ojha S et al., 2018).

The formula applied was: $EF = A(\lambda_{max}) \times d$ (1)

The results, expressed as mean values of two samples of *Paederia foetida* extract in both solvents, are represented in Table 1. According to Table 1 and Figure 2, the EF in ethanol was much superior to that in acetone. Polyphenols and phenolic acid derivatives like scopoletin, quercetin, rutin, vanillin, catechin, etc (absorption in the 310-330 nm region) prefer the more polar and protic ethanol over acetone for their extraction, as also suggested from their ~10 times higher EF in EtOH than acetone. Also, the extraction of carotenoids present in the plant extracts was augmented in the polar EtOH solvent.

Table 1. The absorption maxima λmax (nm) of each plant extract from UV-Vis spectra and the mean values calculated (X ± SD) for extraction factors (EF).

Solvent used	λ_{max} (nm)	EF
Ethanol	328	37.6 ± 0.08
	410	82.8 ± 0.18
	665	29.2 ± 0.11
Acetone	342	3.6 ± 0.008
	408	8.4 ± 0.01
	665	3.05 ± 0.01

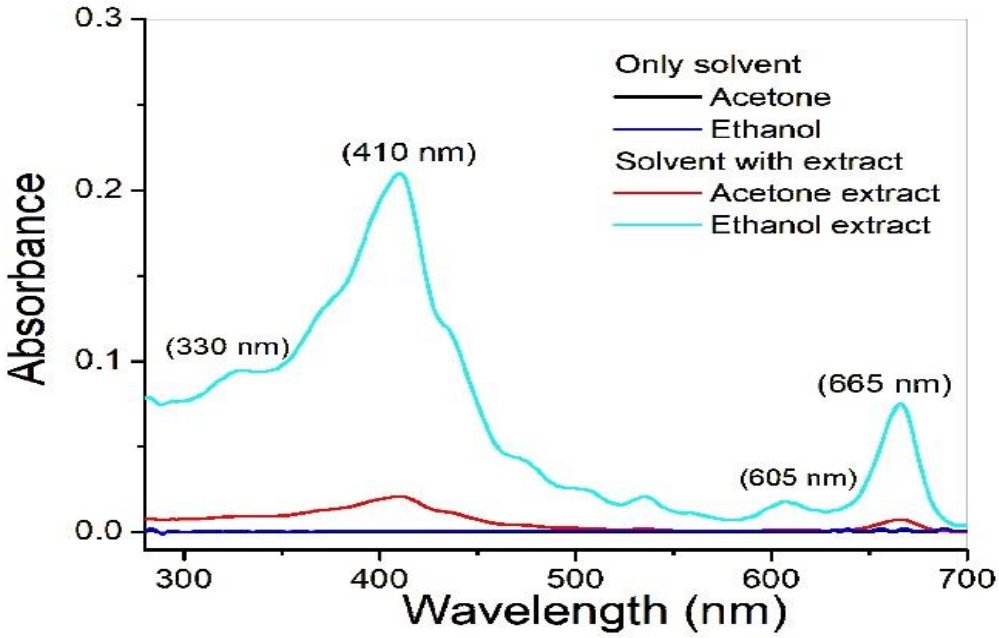

Figure 2. UV-Vis spectra of the acetone extract (Red) in acetone solvent and the ethanol extract (Cyan) in EtOH solvent. The baseline for the two pure solvents is given for reference.

3.2. FTIR studies

The FT-IR spectra (4000-500 cm-1) of the extracts in each solvent were registered, and the specific wave numbers and intensities were considered. The functional groups identification was based on the FTIR peaks attributed to stretching and bending vibrations. Five areas (marked from 1 to 5) (Fig. 3) were identified in the IR domain, and the fingerprint region was localized between 900 and 1400 cm-1.

Vibrations in the region of less than 1000 cm-1 correspond to C–H bending vibrations from carotenoids and terpenoids. Area 5 (1000-1150 cm-1) includes stretching vibrations C–O of glycosides and mono-, oligo- and carbohydrates, with signals at 1032, 1052, 1152 cm-1, while area 4 (1250-1450 cm-1) corresponds to stretching vibrations of carbonyl C-O, O-H bendings, and C-C stretchings from phenyl groups (Dutta PP et al., 2023; Kaushik N et al., 2023; Osman H et al., 2009) Area 3 is a complex one (1580-1760 cm-1) mainly including N-H bending vibrations, C=O stretchings (aldehydes and ketones, esters as well free glycerides (1730 cm-1) and the aromatic domain (Kaushik N et al., 2023; Osman H et al., 2009) Area 2 (2800-3000 cm-

1), corresponds to C-H stretching vibrations specific to CH3- and CH2- from lipids, methoxy derivatives, C-H (aldehydes). Area 1 (3350-3600 cm-1) corresponds to stretching vibrations of -OH groups from water, alcohols, phenols (3342 cm-1), carbohydrates, and peroxides. (Dutta PP et al., 2023; Kaushik N et al., 2023; Osman H et al., 2009). In the ethanolic extract, IR-peaks in the domain 1050-1100 cm-1 and 3340 cm-1 have much higher integrated IR-absorption areas, suggesting a greater specific concentration of the functional groups in the solvent, which remains in agreement with other spectroscopic determinations. Notably, for therapeutic reasons, it could be considered that evaporated ethanol extracts would provide higher concentrations of bioactive molecules from these plants.

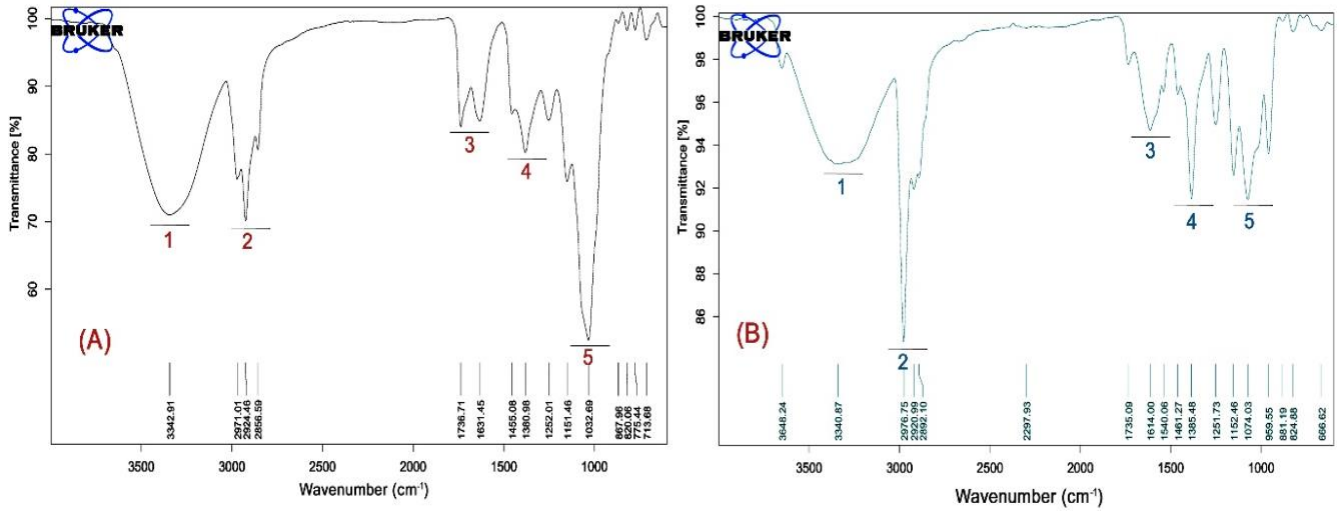

Figure 3. FTIR spectra of the P. foetida extract in (A) Ethanol and (B) Acetone solvent.

3.3. Antibacterial Activity

To address the antibacterial effects of leaves extracts of *Paederia foetida* zone of growth inhibition of *E.coli* bacteria has been observed. Figure 4 showed a significant zone of growth inhibition upon treatment of different concentrations of ethanolic leaves extract of *Paederia foetida* into wells of agar plates (10, 30, 50, 70, 80, 100 µg/ml). The diameters of the zone of inhibition were increased upon increasing the concentration of ethanolic leaf extract (12 mm, 15 mm, 17 mm, 19 mm, 21 mm, 23 mm, respectively). But in the case of untreated and DMSO treatment, no zone of inhibition was observed. The maximum zone of growth inhibition was found at a concentration of 100 µg/ml of ethanolic leaves extract is 23 nm by using the well diffusion method.

On the other hand, *E. coli* bacteria were treated with same concentrations of ethanolic extract of *Paederia foetida* in nutrient broth culture; found decrease of optical density of bacterial culture. Treatment with 10 µg/ml of ethanolic leaves extract with *E. coli* bacteria showed negligible growth inhibition, but upon increasing the concentration (30-100 µg/ml) of ethanol extract, bacterial growth gradually decreased, and at a concentration of 100 µg/ml, almost complete bacterial growth inhibition occurred (Fig. 5).

Similarly, the acetone leaves extract of *Paederia foetida* also revealed significant growth inhibition against *E. coli* bacteria by the well diffusion method (Fig. 6). The concentration of acetone leaves extract of *Paederia foetida* from 10 µg/ml to 100 µg/ml, the diameter of the zone of inhibition increases from 13 nm to 20 nm. Simultaneously, when *E.*coli bacteria were treated with a varied number of concentrations, such as 10, 30, 50, 70, 80, and 100 µg/ml of acetone leaves extract of *Paederia foetida, a* similar incident was found in ethanol extract. The bacterial growth gradually decreased, and upon treatment with

100 µg/ml of acetone extracted from *Paederia foetida* leaves drastically reduced the OD value of the bacterial culture and almost complete bacterial growth inhibition was observed (Fig. 7).

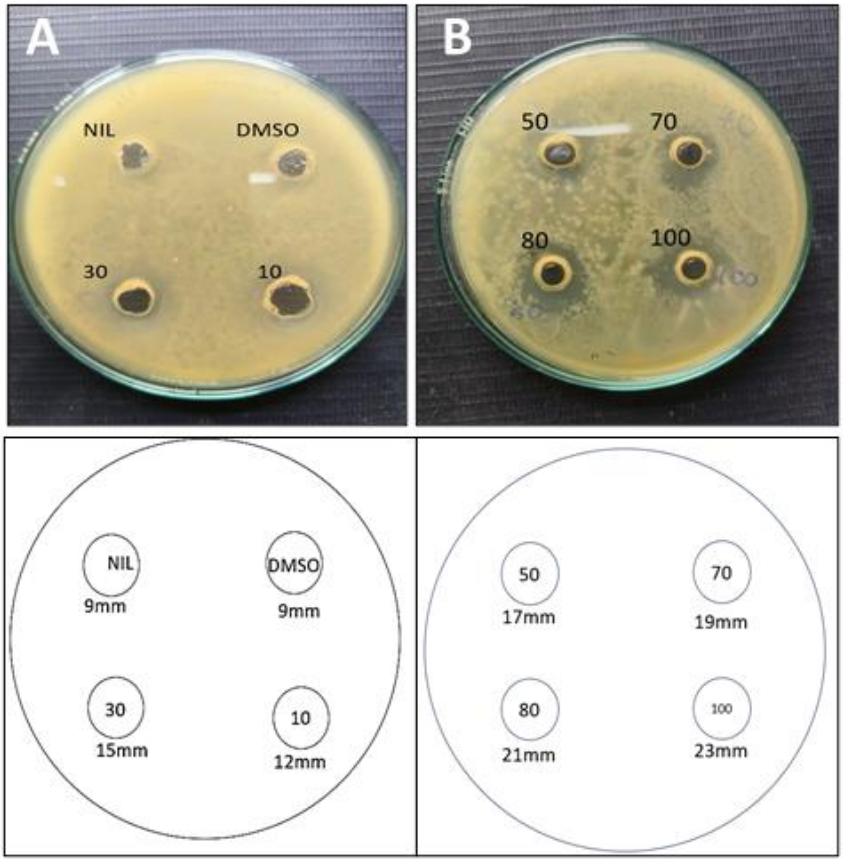

Figure 4. Zone of inhibition after treatment of ethanolic leaf extract of *Paederia foetida* (0-100 µg/ml) with *E. coli* bacteria by the well diffusion method. Corresponding downwards are represented the measurements of the diameter of zone of inhibition by the ethanolic leaf extract of *Paederia foetida* at a lower dose (0-100 µg/ml).

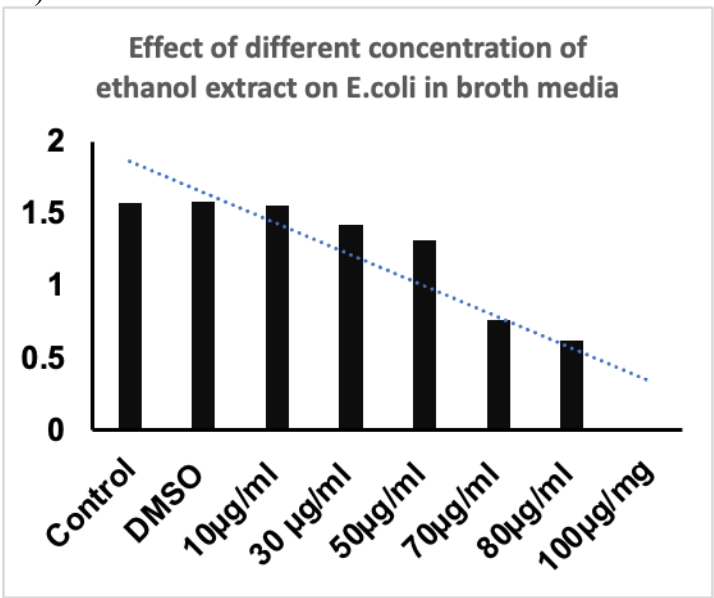

Figure 5. Optical density of bacterial culture upon treatment with different concentrations of ethanolic leaf extract of *Paederia foetida*.

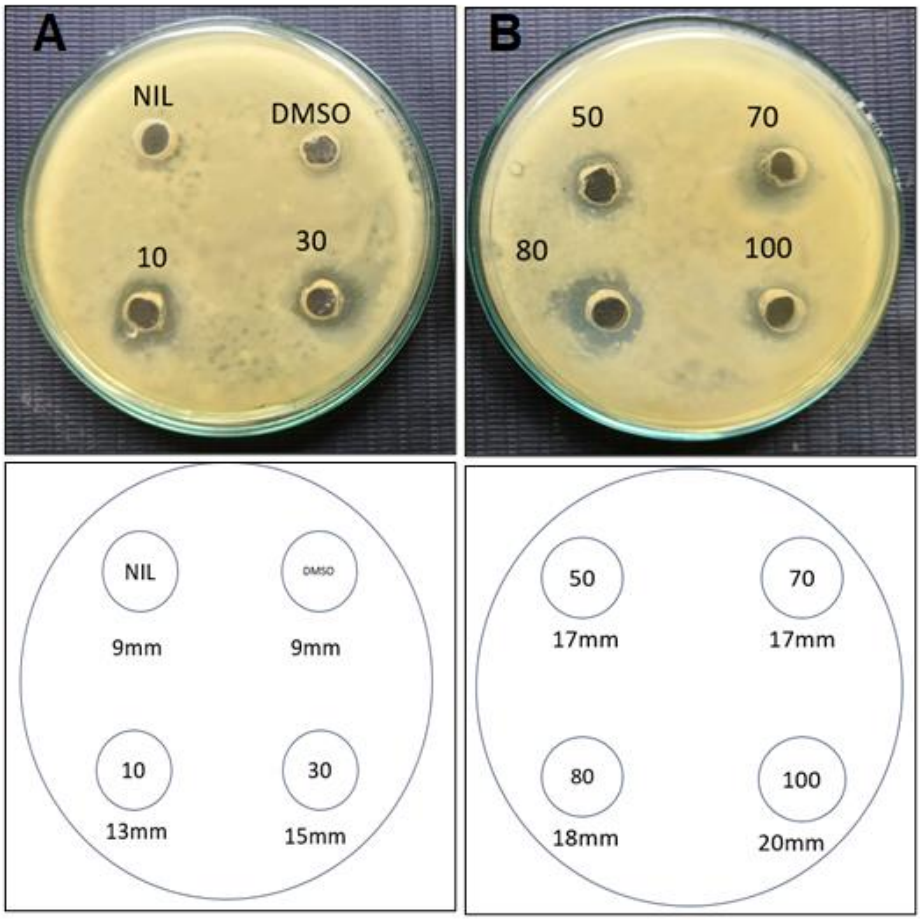

Figure 6. Zone of growth inhibition after treatment of the acetone leaf extract of *Paederia foetida* (0-100 µg/ml) with *E. coli* bacteria by the well diffusion method. Corresponding downwards are represented the measurements of the diameter of the zone of inhibition by the acetone leaf extract of *Paederia foetida* at a lower dose (0-100 µg/ml).

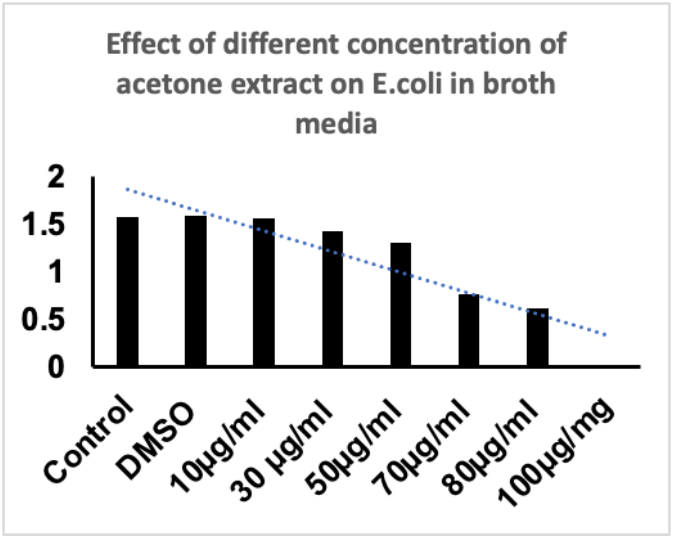

Figure 7. Optical density of bacterial culture upon treatment with different concentrations of acetone leaf extract of *Paederia foetida*.

Both ethanolic and acetone leaf extracts of *Paederia foetida* demonstrated antibacterial activity against the gram-negative bacterium *Escherichia coli* (*E. coli*). The degree of growth inhibition was comparable between the two extracts. However, ethanol can be preferred for the extraction due to its several advantages: it is cost-effective, polar, easily available, environmentally friendly, less toxic, relatively more soluble, and has a lower boiling point (Khotimah H et al., 2020). The low boiling point of ethanol is particularly beneficial during the solvent removal process, as it allows for separation at lower temperatures, thereby minimizing the risk of degrading heat-sensitive bioactive compounds (Jeyaseelan CE et al., 2012). Most importantly, ethanolic extraction of *Paederia foetida* leaves yields maximum bioactive molecules. The observed antibacterial activity of the leaf extracts is likely due to the presence of various bioactive molecules. Previous studies have shown that *Paederia foetida* contains a wide range of phytochemicals, including saponins, tannins, phenols, flavonoids, terpenoids, cardiac glycosides, alkaloids, and reducing sugars (Jeyaseelan CE et al., 2012). Our UV-VIS and FTIR analysis confirmed the presence of phenolic compounds, flavonoids, glycosides, and possibly terpenoids or carotenoids in both leaf extracts. These compounds are believed to contribute to the plant's antibacterial properties. Nevertheless, the potential mechanisms by which these bioactive compounds exert antibacterial effects vary. For example, alkaloids may inhibit protein and nucleic acid synthesis, disrupt bacterial cell membrane function, inhibit ATP synthesis, and block efflux pump activity on bacterial membranes (Yan Y et al., 2021). Flavonoids are known to interfere with bacterial energy metabolism, inhibit nucleic acid synthesis, and disrupt cytoplasmic membrane function by inhibiting ATPase and phospholipase enzyme binding, thereby compromising membrane permeability (Xie Y et al., 2014). Additionally, saponins, terpenoids, and steroids may exhibit antibacterial activity by reducing membrane permeability or by interacting with proteins in the lipopolysaccharide layer of gram-negative bacteria (Yunita M, 2023). However, the mode of action of acetone-extracted phytochemicals needs to be explored.

4. Conclusions

UV-VIS and FTIR studies confirmed the presence of polyphenolic compounds, carotenoids, and terpenoids in the leaf extract of *Paederia foetida.* IR-peaks of ethanolic extract at the domain 1050-1100 cm-1 and 3340 cm-1 indicated higher integrated IR-absorption areas and suggested a greater specific concentration of the functional groups in the solvent, which remains in agreement with other spectroscopic determinations. Furthermore, evaporated ethanol extracts would provide higher concentrations of bioactive molecules from these plants that are therapeutic in nature. And both ethanol and acetone leaves extract revealed strong growth inhibition activity against Gram-negative *E. coli* bacteria.

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