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Evaluation of Antibacterial Activity of *Piper betle* L., *Lawsonia inermis* L. and *Mangifera indica* L. Leaf Ethanol Extracts

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Abstract

Crude plant extracts comprise extensive composition of active chemical constituents serving broad spectrum pharmacological functions. These bioactive elements can be extracted and employed towards multitudinous applications from healthcare to ecological sectors. The present study was carried out to investigate the antibacterial potential of ethanol extracts prepared by cold maceration method from *Piper betle* L., *Lawsonia inermis* L. and *Mangifera indica* L. dried leaves against seven different bacterial pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus spp.*, *Streptococcus mutans, Enterococcus spp.* and *Klebsiella pneumoniae.* Antibacterial activity was evaluated by agar well diffusion method for the crude ethanol extracts and for the ethanol extracts prepared at six different concentrations such as 25 µg/mL, 50 µg/mL, 150 µg/mL,

300 µg/mL, 500 µg/mL and 1000 µg/mL. The experiment was performed in triplicate(n = 3) and results are expressed in mean ± standard deviation. Diameter of zone of inhibition was measured in mm.

Keywords: Antibacterial activity, Ethanol extracts, Concentrations, *Piper betle* L., *Lawsonia inermis* L., *Mangifera indica* L., Leaves, Inhibition zone.

Introduction

Plants contain diversified distribution of chemical constituents that are synthesized through various metabolic pathways and can be extracted using suitable solvents following appropriate extraction methodologies. Crude plant extracts thus derived serve as vital pharmacological repositories exerting numerous medicinal properties also, employed as therapeutic agents in treating infections caused by bacteria, virus, fungi, parasites etc. thereby serving different pharmaceutical functions **(Álvarez-Martínez, F. J. *et al.,* 2025).** Infectious diseases caused by bacterial pathogens pose serious risk and concern worldwide due to their antibiotic resistance and ability to reemerge **(Nouhaila Zouine *et al.,* 2024).** Medicinal plants are known for their historical usage as medicaments in curing various pathological conditions and contagious diseases **(Bereksi MS *et al.,* 2018).** Plant derived drugs are preferred globally in treating various ailments and are known to display different mechanisms in inhibiting the microbial growth and transmission **(Van Galen, E. *et al.,* 2014; McMurray, R. L. *et al.,* 2020).** Antimicrobial compounds extracted from plants exhibit innumerable properties as a result of the combined effectiveness from secondary metabolites such as phenols, flavanoids, glycosides, alkaloids, tannins, coumarins etc. distributed in various plant parts. These bioactive components act in a synergistic way increasing their bioavailability, rate of resorption, solubility thereby, controlling adverse reactions and minimizing the toxic effects **(Wagner, H. *et al.,* 2009;Vaou, N. *et al.,* 2021).** Phytochemical compounds are also utilized in treating cold, cholera, bronchitis, dysentery, cough, diarrhoea etc. **(Joshi, B. *et al.,* 2009).**It has been reported that the rate of emergence of pathogenic diseases with drug resistance is becoming rapidly higher than the rate of production and usage of medications. Naturally derived antibiotic compounds are preferred over synthetic drugs since, the latter could pose side effects and contribute towards bacterial resistance while, natural compounds are known to be safe and effective **(Alaa' Turki Monawer and Ismaeil Mohammed Abdulkahar Mammani., 2023).**Thus, there arises a need for research and development of antibacterial drugs that could aid in combating the diseases **(Yan Y *et al.,* 2021; Modarresi Chahardehi A *et al.,* 2012; Modarresi Chahardehi A., 2023; Barati, M., and Modarresi Chahardehi, A., 2024).**This study aims to evaluate the antibacterial efficacy of ethanol extracts prepared at different concentrations (25 µg/mL to 1000 µg/mL) in comparison to the crude ethanol extracts derived from the leaves of *Piper betle* L., *Lawsonia inermis* L. and *Mangifera indica* L. against various bacterial pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus spp.*, *Streptococcus mutans, Enterococcus spp.* and *Klebsiella pneumoniae.*

Materials and Methods

Preparation of Ethanol extracts: Ethanol extracts were prepared from the dried and powdered leaves of *Piper betle* L., *Lawsonia inermis* L. and *Mangifera indica* L. following cold maceration method in the ratio of 1:10 (10 grams of dried leaf samples in 100 mL of ethanol). Extraction procedure and methodologies are reported in our previous papers **(Leela K and Singh A. R. J., 2020; Karunakaran L and Singh A. R. J., 2025).**

Assessment of antibacterial activity of ethanol extracts: Antibacterial activity of *Piper betle* L., *Lawsonia inermis* L. and *Mangifera indica* L. leaf ethanol extracts were determined against seven

bacterial pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus spp.*, *Streptococcus mutans, Enterococcus spp.* and *Klebsiella pneumoniae* by agar well diffusion method using Muller Hinton agar medium. Ethanol extracts prepared at six different concentrations such as 25 µg/mL, 50 µg/mL, 150 µg/mL, 300 µg/mL, 500 µg/mL, 1000 µg/mL along with crude ethanol extracts were dispensed into the wells and incubated for about 24 hours to assess the zone of inhibition. Antibacterial efficacy of crude ethanol extracts and ethanol extracts at different concentrations were compared. Diameter of inhibition zone was measured in mm. Experiment was carried out in triplicate and values are expressed in mean ± standard deviation **(Leela K and Singh A. R. J., 2020; Acharjee, M.*et al.*, 2022).**

Results

Antibacterial activity of ethanol extracts from *Piper betle* L., *Lawsonia inermis* L. and *Mangifera indica* L. dried leaves were assessed by means of agar well diffusion method against seven bacterial pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus spp.*, *Streptococcus mutans, Enterococcus spp.* and *Klebsiella pneumoniae.* Crude ethanol extracts and ethanol extracts prepared at different concentrations such as 25 µg/mL, 50 µg/mL, 150 µg/mL, 300 µg/mL, 500 µg/mL and 1000 µg/mL were determined for their inhibitory efficacy against different bacterial organisms. The experiment was carried out in triplicate (n=3) and results are expressed in Mean ± Standard deviation. The diameter of inhibition zone was measured in mm and the results are tabulated **(Tables 1-3).** The plates showing corresponding inhibition zones are given **(Figures 1 – 3)**

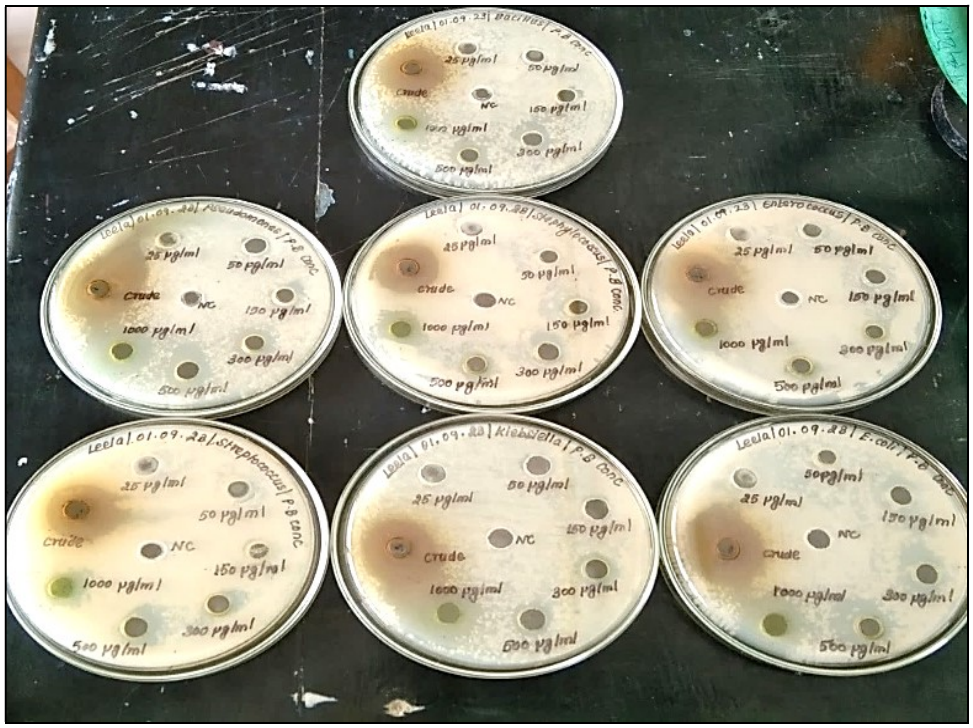

Figure 1: Antibacterial activity of *Piper betle* L. ethanol extracts

Table 1: Antibacterial activity of *Piper betle* L. ethanol extracts

Piper betle L.	Zone of Inhibition (in mm)						
	Concentration (µg/mL)						
	25	**50**	**150**	**300**	**500**	**1000**	**Crude**
Staphylococcus aureus	-	4 ± 0	8 ± 0	10 ± 0	12 ± 0	15.8 ± 0.7	19.6 ± 0.5
Streptococcus mutans	2 ± 0	4 ± 0	4.3 ± 0.5	10 ± 0	14 ± 0	14 ± 0	29.3 ± 0.5
Escherichia coli	8.5 ± 0.8	10 ± 0	14 ± 0	20 ± 0	22 ± 0	22 ± 0	30 ± 0
Klebsiella pneumoniae	-	2 ± 0	4.3 ± 0.5	8 ± 0	12 ± 0	22 ± 0	34 ± 0
Pseudomonas aeruginosa	2 ± 0	4 ± 0	8 ± 0	12 ± 0	18 ± 0	24 ± 0	43.8 ± 0.5
Bacillus spp.	2 ± 0	2.6 ± 0.5	6 ± 0	14 ± 0	16 ± 0	20 ± 0	24 ± 0
Enterococcus spp.	2 ± 0	3.6 ± 0.5	10 ± 0	11.6 ± 0.5	12 ± 0	13.8 ± 0.2	28 ± 0

Piper betle L. ethanol extracts displayed higher antibacterial activity against *Pseudomonas aeruginosa* with 24 ± 0 mm at a concentration of 1000 µg/mL and minimum inhibition was observed against *Streptococcus mutans*, *Pseudomonas aeruginosa, Bacillus spp.*and *Enterococcus spp.* with 2 ± 0 mm at a concentration of 25µg/mL while, no inhibition zone was observed against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Crude ethanol extracts exhibited higher inhibition zone of 43.8 ± 0.5 mm against *Pseudomonas aeruginosa* and minimal inhibition against *Staphylococcus aureus* with 19.6 ± 0.5 mm**(Table 1).**

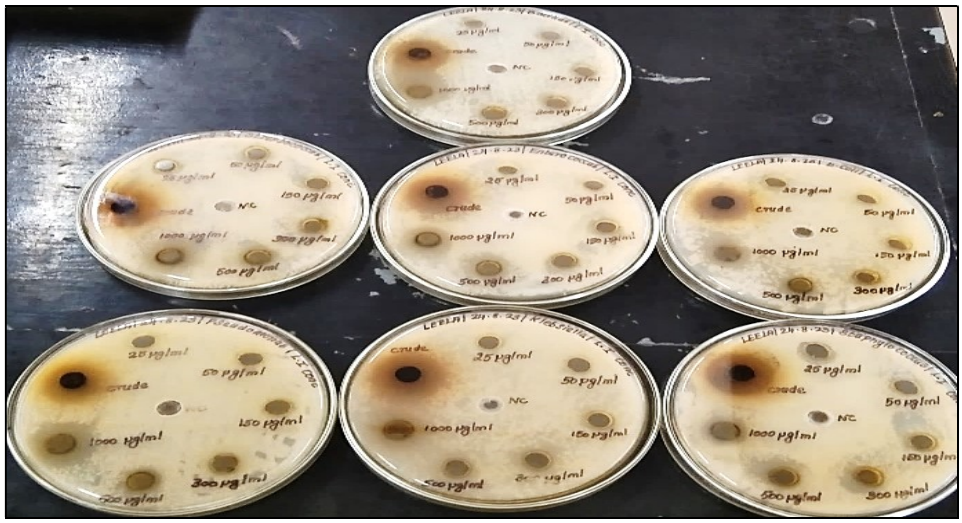

Figure 2: Antibacterial activity of *Lawsonia inermis* L. ethanol extracts

Table 2: Antibacterial activity of *Lawsonia inermis* L. ethanol extracts

*Lawsonia inermis*L.	Zone of Inhibition (in mm)						
	Concentrations (µg/mL)						
	25	50	150	300	500	1000	Crude
Staphylococcus aureus	2 ± 0	4.3 ± 0.5	6 ± 0	7.8 ± 0.2	12 ± 0	14 ± 0	20 ± 0.2
Streptococcus mutans	2 ± 0	6 ± 0	10.3 ± 0.5	14 ± 0	18 ± 0	24 ± 0	40 ± 0
Escherichia coli	2 ± 0	4 ± 0	4.1 ± 0.2	10 ± 0	12 ± 0	22 ± 0	24 ± 0.5
Klebsiella pneumoniae	2 ± 0	3 ± 0	6 ± 0	12 ± 0	16 ± 0	20 ± 0.2	24 ± 0
Pseudomonas aeruginosa	1.6 ± 0.5	2.3 ± 0.5	4 ± 0	6 ± 0	8 ± 0	16 ± 0	24 ± 0
Bacillus spp.	1.6 ± 0.5	5.8 ± 0.2	7.8 ± 0	8 ± 0	10 ± 0	18 ± 0	22 ± 0
Enterococcus spp.	3.6 ± 0.5	6 ± 0	8 ± 0	8.8 ± 0.2	13.6 ± 0.5	18 ± 0	20 ± 0

Lawsonia inermis L. displayed higher antibacterial activity against *Streptococcus mutans* with 24 ± 0 mm at a concentration of 1000 µg/mL and minimum inhibition was observed against *Pseudomonas aeruginosa* and *Bacillus spp.*with 1.6 ± 0.5 mm at a concentration of 25µg/mL while, crude ethanol extracts exhibited higher inhibition of 40 ± 0 mm against *Streptococcus mutans* and minimal inhibition against *Staphylococcus aureus* with 20 ± 0.2 mm and *Enterococcus spp.* with 20 ± 0 mm**(Table 2).**Ethanol extracts from *Lawsonia inermis* L. displayed effective inhibition against all bacterial pathogens at all concentrations.

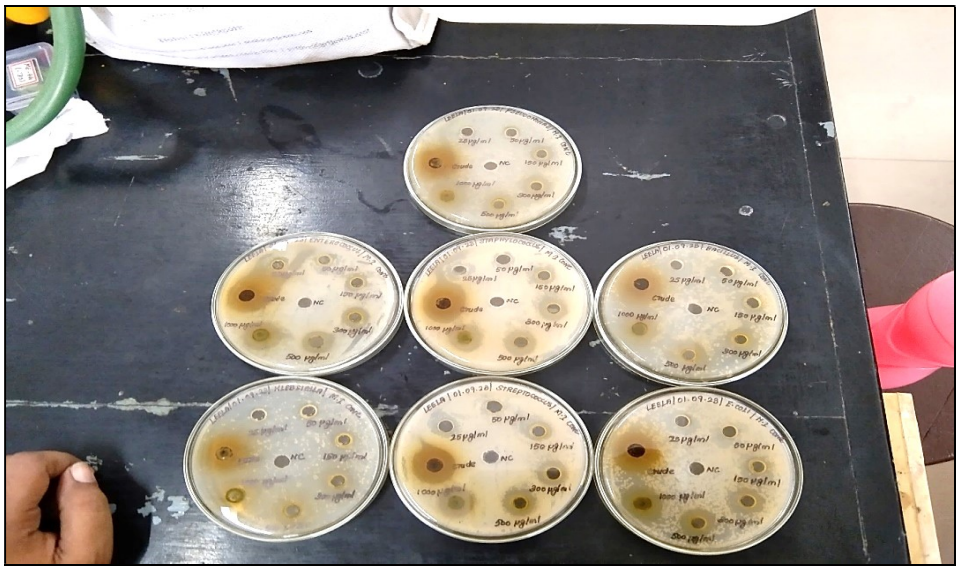

Figure 3: Antibacterial activity of *Mangifera indica* L. ethanol extracts

Table 3: Antibacterial activity of *Mangifera indica* L. ethanol extracts

Mangifera indica L.	Zone of Inhibition (in mm)						
	Concentrations (µg/mL)						
	25	50	150	300	500	1000	Crude
Staphylococcus aureus	6 ± 0	6 ± 0	12 ± 0	15.6 ± 0.5	20 ± 0	24 ± 0	25.6 ± 0.5
Streptococcus mutans	4 ± 0	5.8 ± 0.3	7.8 ± 0.2	10 ± 0	12 ± 0	12 ± 0	26.1 ± 0.2
Escherichia coli	8 ± 0	12 ± 0	12 ± 0	14 ± 0	16 ± 0	20 ± 0	22 ± 0
Klebsiella pneumoniae	6.3 ± 0.5	10 ± 0	12 ± 0.2	16 ± 0	18 ± 0	20 ± 0	26 ± 0
Pseudomonas aeruginosa	2 ± 0	2 ± 0	4 ± 0	7.6 ± 0.5	12 ± 0	20 ± 0	23.8 ± 0.2
Bacillus spp.	10 ± 0	12 ± 0	15.6 ± 0.5	20 ± 0	24 ± 0	30 ± 0	34 ± 0
Enterococcus spp.	4.3 ± 0.5	10 ± 0	10.8 ± 0.7	12 ± 0	14 ± 0	18 ± 0	36 ± 0

Mangifera indica L. displayed higher antibacterial activity against *Bacillus spp.* with 30 ± 0 mm at a concentration of 1000 µg/mL and minimum inhibition was observed against *Pseudomonas aeruginosa* with 2 ± 0 mm at a concentration of 25µg/mL while, crude ethanol extracts exhibited higher inhibition of 36 ± 0 mm against *Enterococcus spp.* and minimal inhibition against *Escherichia coli* with 22 ± 0 mm **(Table 3).**Ethanol extracts from *Mangifera indica* L. displayed effective inhibition against all bacterial pathogens at all concentrations.

Discussion

Ethanol extracts at the concentration of 1000 µg/mL displayed greater inhibition zone while, least inhibition zone was observed at the concentration of 25 µg/mL in all three plant samples. Comparatively, crude ethanol extracts displayed potentially higher antibacterial activity against all bacterial organisms than the extract at different concentrations since, crude ethanol extracts contain concentrated amounts of bioactive components compared to the diluted extracts. Study by **Hemeg, H. A. *et al.*, 2020** has reported antimicrobial activity of *Psidium guajava, Ziziphusspina christi, Olea europaea* L., *Morusalba* L. and *Salvia officinalis* leaf ethanol extracts against *E. coli, S. aureus B. cereus*, *Pasteurella multocida*, *M. gallisepticum* and *Salmonella enteritidis* by agar disc diffusion method of which, all the plant extracts were found to have demonstrated effective antibacterial activity however, *Psidium guajava* leaf ethanol extracts revealed strong antibacterial activity against *Mycoplasma gallisepticum* which was reported to have not been displayed by the other plant samples. Antibacterial activity of *Emblica officinalis* Gaertn seed methanol extracts were evaluated against *Escherichia coli, Proteus vulgaris* and *Klebsiella pneumonia* wherein, maximum activity was reported against *Escherichia coli,* minimal activity against *Proteus vulgaris* and no inhibition was observed against *Klebsiella pneumoniae* **(G Singh, P. *et al.*, 2022).** A Study has reported antibacterial activity of *Azadirachta indica* leaf ethanol and aqueous extracts on *Staphylococcus aureus* by disc diffusion method wherein, ethanol extracts were found to have demonstrated greater antibacterial activity compared to the aqueous extracts **(Bright Chukwudi Francis., 2023).**

Conclusion

Antibacterial activity of *Piper betle* L., *Lawsonia inermis* L. and *Mangifera indica* L. leaves ethanol extracts were demonstrated in this study wherein, ethanol extracts from all three plant samples displayed effective inhibition against all pathogenic organisms at all the tested concentrations however, concentrated crude ethanol extracts exhibited greater inhibitory activity compared to the diluted extracts. Further studies on these extracts could also widen the scope for determining their potential usage in numerous therapeutic applications.

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