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Original Research Article

Antimicrobial Studies of Ethanol Extract of *Persicaria odorata* (Lour sojak) Against Some of the Commonly Isolated Microorganisms

Ningthoujam Diana Devi[1], Laishram Babycha[2], Debashree Ningthoujam[3], Chongtham Urvashi[4], Ningombam Minita[5]

[1]PGT Department of Pharmacology, Jawahar Lal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur, India

[2]Associate Professor, Department of Pharmacology, JNIMS, Imphal, Manipur, India

[3]Assistant Professor, Department of Pharmacology, JNIMS, Imphal, Manipur, India

[4]Professor, Department of Microbiology, JNIMS, Imphal, Manipur, India

[5]Associate Professor, Department of Community Medicine, JNIMS, Imphal, Manipur, India

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Corresponding Author: Debashree Ningthoujam

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Abstract:

The growing prevalence of multidrug-resistant microorganisms, driven by the overuse and misuse of antibiotics, poses a major global public health challenge by reducing the effectiveness of existing drugs. This study evaluated the antibacterial activities of Persicaria odorata (Phakpai) extracts to assess their potential as natural therapeutic alternatives. Antibacterial activity was tested against Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Streptococcus pneumoniae using the agar disc diffusion method at dose of 150, 300, and 600 µg. Gentamicin and ampicillin (10 µg) served as positive controls. The ethanolic extract at 600µg demonstrated the highest antibacterial efficacy, producing inhibition zones of 9.93 mm against E. coli and K. pneumoniae, 9.50 mm against P. aeruginosa, and 16.27 mm against S. aureus. Ampicillin inhibited E. coli, and S. aureus, but not P. aeruginosa and K. pneumoniae, while gentamicin was effective against all tested organisms. The superior antimicrobial potency of the ethanolic extract is likely attributed to its higher solubility for bioactive compounds, such as phenolics and alkaloids, which enhance bacterial cell wall permeability, particularly in Gram-positive strains. These findings suggest that P. odorata is a promising source of natural antimicrobial and antioxidant compounds and may provide a foundation for developing novel agents to combat antibiotic-resistant pathogens.

Keywords: Persicaria odorata, antioxidant, antibacterial, multidrug resistance, ethanolic extract, natural therapeutics.

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Introduction

In recent decades, resistance to synthetic antibiotics and antibacterial agents has become a major public health concern, largely due to their indiscriminate usage. [1] Moreover, the lack of newly developed antibacterial drugs has become a significant issue for all researchers globally. Each year, more than 13 million deaths globally are attributed to the emergence of new infectious diseases or the resurgence of previously controlled pathogens. [2] Herbal medicines are gaining special attention due to the increased concerns about safety, availability, and negligible side effects as compared to synthetic medicines. [3] Therefore, finding and identifying medicinal plants with the potential to combat multidrug-resistant microbes could represent a promising and sustainable solution. Interestingly, plants are reported to possess the ability to produce primary and secondary metabolites that play a crucial role in the development and in defence mechanisms against pests and pathogens before they cause serious damage. Plant-based ethnic foods are rich in nutrients and valuable bioactive chemical compounds, making them valuable, potent sources for the development of novel therapeutic agents. [4] Presence of various active compounds is reported in the P. odorata extracts, such as flavonoids (quercetin, Smyricetin, and gallic acid) and phenolic compounds.[5] They are known to have antioxidant and medicinal properties. [6,7] However, limited studies have been conducted on Persicaria odorata a medicinal plant extensively found in the tropical warm and humid regions. Therefore, P. odorata represents a potential candidate for screening antibacterial properties for potential drug development to treat various ailments.

Aims and Objectives: To evaluate the antimicrobial effect of ethanol extract of Persicaria odorata (Lour

Sojak) against some commonmy isolated microorganisms

Materials and Methods

The study was conducted in the Department of Pharmacology and Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal.

Collection and Identification of Persicaria odorata: A bulk amount of fresh P. odorata plant samples were collected from the local market during the month of September 2024. Morphological and taxonomical identification was carried out (by guest lecturer Dr. Yumkham Sanatombi Devi) in the Department of Botany, School of Life Sciences, Manipur University. A plant sample was deposited at the laboratory herbarium and allocated with voucher no. 001468MUMP.

Preparation of Plant Extracts: Ethanol extracts of leaves of P. odorata was prepared by using Soxhlet apparatus. Freshly collected leaves of P. odorata were thoroughly washed with cold water and dried in shade at the room temperature. The dried leaves were finally powdered with the help of electric mixture grinder. 50 grams of the powder of leaves was extracted with 500 ml of ethyl acetate by using Soxhlet apparatus for 16 hours. The crude extract obtained was filtered using Whatman filter paper and filtrate was evaporated. The extract was collected in sterile glass petridish and finally stored in air tight glass containers. In this way, the procedure was repeated several times to yield 20 gm of the extract and preserved in sterile container at 4 C, which was used as test material throughout the study.

Bacterial Strains: Gram-negative bacteria such as Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 27553), Pseudomonas aeruginosa (ATCC 25923) and Gram positive bacteria Staphylococcus aureus (ATCC 25922) from the American Type Culture Collection were all employed as the four bacterial strains. The ATCC bacterial strains were provided by Microbiology Department JNIMS, Imphal, Manipur.

Antibiotics and Media: Antibiotics used were Ampicillin 10 and Gentamicin 10. Media used were nutrient agar and Mueller-Hinton Agar

Preparation of bacterial strains: The bacterial strains (K. pneumoniae, E. coli, P. aeruginosa and S. aureus) were revived by streaking on freshly prepared nutrient agar media. The cultures were incubated at 37°C for 24 hours by using the incubator. A single colony from the overnight culture was used to prepare peptone broth culture using broth media. The inoculated cultures were incubated at 37°C for 24 hours.

Preparation of bacterial culture: The test organisms i.e. Staphylococcus aureus, Klebsiella pneumonieae, E.coli and Pseudomonas aeruginosa were inoculated at peptone water for 3-4 hours.

Mueller Hinton Agar Petri dishes for culture-sensitivity were prepared by standard procedure 5-6 mm depth in 90mm diameter petriplate. Isolates were seeded on Mueller Hinton agar plates by a sterilised cotton swab and a lawn culture were prepared.

Preparation of plant extract disc: The extract discs (5 mm diameter) were prepared by using Whatman filter paper no 1. First a solution of ethanol extract was made by dissolving the extracts in absolute alcohol. The concentration of the solution was kept at 30mg/ml. The solution was stored at 4 C to prevent it from contamination. To prepare 150 µg, 300 µg and 600 µg disc, first 5mm diameter of discs were prepared by punching. Then the discs were sterilised by autoclaving at 121°C, 15 psi for 20 minutes.

Discs of the desired solution was prepared by using a micropipette. 5 µl, 10 µl and 20 µl of solution from ethanol extracts were drawn into the pipette by proper adjustment and put on the discs to make 150 µg, 300 µg and 600µg discs respectively. For control 20 µl of absolute alcohol was put on the filter paper discs for ethanol extract. Then the discs were kept for drying in a hot air oven at 50 C for 1 hour and stored at 4°C to prevent from contaminates and degradation of bioactive compounds.

After drying the discs were set on the inoculated culture plates. For the test of ethanol extract, 150µg, 300µg, 600µg disc along with ampicillin (10 µg), gentamicin (10 µg) and control disc were put in the culture plate. The inoculums were incubated at 370C for overnight and zone of inhibition around the standard antibiotic as well as for extract disc and control discs were measured for antibacterial activity. [8]

Screening of Antibacterial activity: The antibacterial activity for both the aqueous and ethanol extract of the plant was evaluated by following disc diffusion method described by Kirby-Bauer with modifications. Freshly prepared broth culture of each 100 µl of K. pneumoniae, E. coli, P. aeruginosa and S. aureus were spread evenly by using a spreader on the surface of Mueller Hinton agar (MHA) media plates. Plant extracts discs (150 µg, 300 µg, 600 µg) of both aqueous and ethanol extracts, along with 10 µg antibiotic discs of ampicillin and gentamicin as positive control and sterile water as negative control were placed on the plate. The inoculated MHA plate with the antibiotics and plant extract disc were incubated at 37°C for 24 hours by using the incubator. The zone of inhibition was measured to understand the antimicrobial

activities. All the experiments were repeated at least three times with three replicates [9].

Statistical analysis: All the experiments were repeated at least three times with three replicates. The result of the experiments is represented as Mean ± Standard error (SE). Statistical analysis was performed using one-way ANOVA, followed by Bonferroni's post hoc test, where $p < 0.05$ considered as significant difference using IBM SPSS version 27.0.

Results

Antimicrobial activity

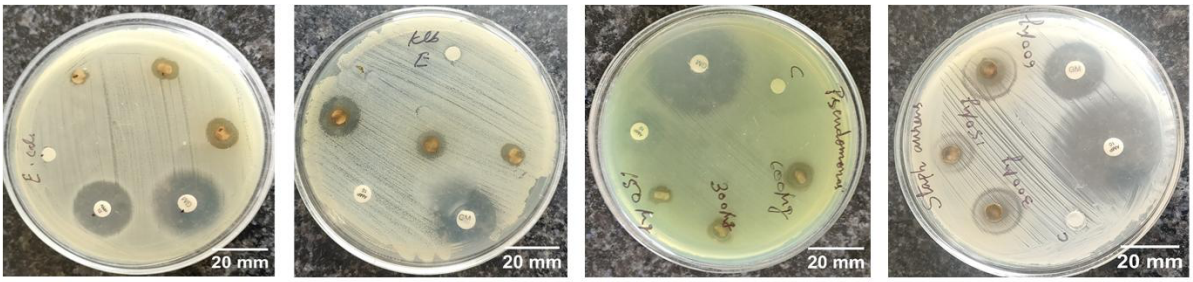

Figure 1: Formation of zone of inhibition by plant extracts (P. odorata) and antibiotic (ampicillin and gentamicin).

In the present study we evaluated the antibacterial property of ethanol extracts of Persicaria odorata using in vitro assay and compared to the known antibiotics such as ampicillin and gentamicin.

The ethanolic extract of P. odorata impregnated discs showed increasing diameter of zone of inhibition in dose dependent manner. And the effects increase when the dose increases from 150 µg to 300 µg and 600 µg, In case of standard antibiotics, gentamicin inhibits all the four bacterial strains at 10 µg and ampicillin inhibit two bacterial strains except K. pneumoniae and P. aeruginosa at 10 µg. The results were expressed as zone of inhibition expressed as Mean ± SEM as shown in (Figure) and (Table)

Table 1: Evaluation of antimicrobial effect of ethanol extract of P. odorata with standard antibiotics Ampicillin and Gentamicin against selected micro-organisms

Extraction solvent/Standard Agent used	Zone of Inhibition (mm) (Mean ± SE)			
	E. coli	K. pneumoniae	P. aeruginosa	S. aureus
Gentamicin	20.33±0.33	20.33±0.33	20.33±0.33	35.67±0.33
Ampicillin	20.67±0.33	-	-	24.33±0.33
150 µg	-	7.43±0.23	-	9.93±0.06
300 µg	7.50±0.28	8.40±0.30	7.47±0.29	12.87±0.13
600 µg	9.93±0.06	9.93±0.06	9.50±0.28	16.27±0.37
F-value	1260.43	1010.54	692.34	1406.93
P-value	<.001	<.001	<.001	<.001
DF	14	14	14	14

Table 2: Comparisons of Antimicrobial efficacies of ethanol extract of Persicaria odorata with standard drug Ampicillin and Gentamicin according to the micro-organisms

Comparison of Antimicrobial		Zone of Inhibition (mm) Mean difference(1-2) ± SE			
1	2	E.Coli	Klebsiella pneumonieae	Pseudomonas aeruginosa	Staphylococcus aureus
GM	150µg	20.33±.352[a]	12.90±.325[a]	20.33±.395[a]	25.73±.391[a]
GM	300µg	12.83±.352[a]	11.93±.325[a]	12.87±.395[a]	22.80±.391[a]
GM	600µg	10.40±.352[a]	10.40±.325[a]	10.83±.395[a]	19.40±.391[a]
Ampicillin	150µg	20.67±.352[a]	-7.43±.325[a]	0.0	14.40±.391[a]
Ampicillin	300µg	13.17±.352[a]	-8.40±.325[a]	-7.47±0.29[a]	11.47±.391[a]
Ampicillin	600µg	10.73±.352[a]	-9.93±.325[a]	-9.50±0.28[a]	8.07±.391[a]

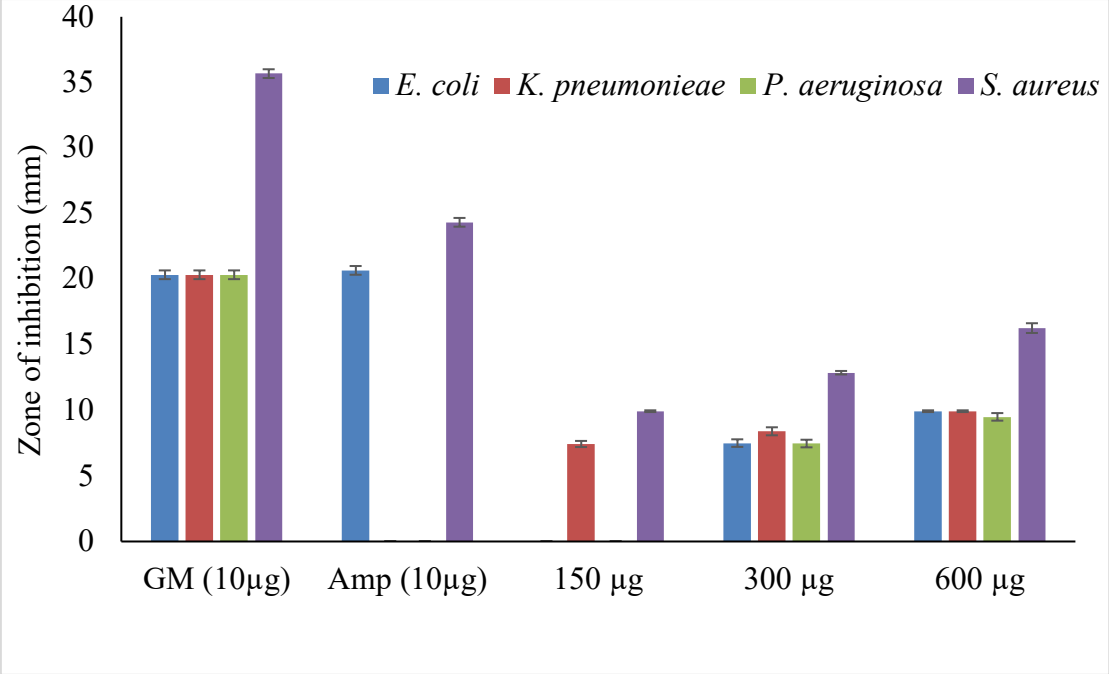

Figure 2: Bar graph representation of antimicrobial activity (zone of inhibition in mm) against E. coli, P. aeruginosa, K. pneumoniae and S. aureus. Each value is represented in Mean ± SE.

Discussion

The present study evaluated the antibacterial effect of ethanol extracts of Persicaria odorata (Lour.) against ATCC strains of E. coli, K. pneumoniae, P. aeruginosa, and S. aureus by using agar disk diffusion method. The antimicrobial activity of the extracts was then compared with that of the standard antimicrobials, gentamicin and ampicillin. Antibacterial activity as indicated by the zone of inhibition is seen with the ethanol extract of Persicaria odorata. The variation in the zone of inhibition suggests the variation in the inhibitory efficacy of the various concentration of the ethanolic extract on the different bacterial strains.

The ethanol extract showed significant inhibitory effects in both the gram positive and gram-negative bacterial strains used, in a dose dependent manner with maximum inhibition seen at 600 µg. There was no inhibition on E. coli and P. aeruginosa at the lowest dose i.e. 150 µg of the ethanolic extract. The most susceptible organism with the most pronounced significant zone of inhibition was S. aureus at each of the concentrations of the ethanolic extract of Persicaria odorata. Gentamicin was found to significantly inhibit all the four bacterial strains used while ampicillin inhibited only E. coli and S. aureus. The inhibition of E. coli and S. aureus with the ethanolic extract of Persicaria odorata was significant compared to both gentamicin and ampicillin. While the inhibition of K. pneumoniae and P. aeruginosa with the ethanolic extract of Persicaria odorata was significant compared to gentamicin.

his suggests that the ethanolic extract of Persicaria odorata has antibacterial efficacy on both the gram positive and gram negative bacterial strains used i.e. E. coli, K. pneumoniae, P. aeruginosa, and S. aureus. This finding is consistent with studies done by Saad R E et al [10] which demonstrated antibacterial efficacy of Persicaria odorata extract on E. coli and S. aureus. It is also consistent with the findings of Ridzuan PM et al [11] who demonstrated antibacterial efficacy of Persicaria odorata extract on E. coli and S. aureus. Yosuf H et al [12] also demonstrated antibacterial activity of Persicaria odorata extract on on E. coli, S. aureus, bacillus subtilis and Salmonella sp. Various other species of Persicaria have also been shown to possesses antibacterial activity against both Gram-negative and Gram-positive bacteria by Lai SM et al [13] and Abubakar MA at al [14]

The finding of antimicrobial activity of P. odorata extracts against the test organisms is a sign that there is likelihood of sourcing alternative antimicrobial agents in this plant for the development of new antibacterial agents to battle various infections associated with susceptible test bacteria utilized in this study and ultimately contribute to reduce the emergence and spread of resistant infections.

Conclusion

Overall, the study supports the traditional medicinal use of P. odorata and highlights its potential as a natural source of antimicrobial and antioxidant agents. Future research should focus on isolating and characterizing the active compounds responsible for these effects, determining their mechanisms of

action, and conducting in vivo studies to evaluate their therapeutic efficacy and safety for potential pharmaceutical applications.

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