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# Evaluation of Piper betle leaf's antibacterial effectiveness against pathogenic microorganisms

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**Abstract:** Multidrug-resistant microorganisms pose challenges for human health, particularly in oral cavity, causing cancer and deadly diseases. Modern generation seeks eco-friendly alternatives to existing chemically synthesized drugs. Piper betle, a mysterious herb, has potential to treat microorganism-induced diseases through its bioactive compounds and metabolites. Piper betle leaves are used as oral refresheners, attracting research interest for antibacterial properties. Dissolved leaves in Methanol, Ethanol, and Aqueous, recovered compounds used as antibacterial agents. Four pathogenic bacteria were identified using Agar Well Diffusion technique. Piper betle leaves showed maximum inhibition zones against Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, and Streptococcus mutant. The aqueous fraction demonstrated extensive activity against Streptococcus mutant and Escherichia coli, with a clear zone of inhibition of 25.66±1.52mm compared to the positive control. Research reveals a novel bio-remedy using piper betle leaves as an edible, herbal, and eco-friendly component. These leaves have remarkable antibacterial potential, potentially treating diseases like dental caries, gingivitis, oral staph, and oral cancer.

**Key Words:** *Piper betle* leaf, Antibacterial potentiality, Solvent extraction, Oral pathogenic bacteria, Biomedicine, Bangla Leaves,etc

**Introduction:** Restorative plants are valuable therapeutics due to their ability to increase safe microorganisms and prevent new infections [1]. Plants contain bioactive compounds that can be used in medicine, agriculture, and industries. Utilizing these compounds as therapeutic agents is a significant area of biomedical and normal product experimentation [2,3]. Piper species are effective restorative plants used for treating common diseases like stomach throb and rheumatoid joint pain. Their bioactive composites contribute to their viability. Piper betle leaves have been shown to be viable against certain disease-causing microbes, but their components are yet to be explained [4]. Piper betle extracts are widely used in treating various diseases due to their cell reinforcement, anticancer, and hypersensitivity properties. Originating in India, this plant belongs to the Piperaceae family and

has over 2000 species [5]. Piper betle species' bioactive metabolites and crude have clinical significance in treating harmful and non-malignant diseases, showing potential as anticancer, antitumor, and antimicrobial agents [6]. Piper betle species contain alkaloids like guineensine, piperine, chabamide, piperlongumine, and pellitorine, which inhibit disease cell growth and apoptosis. This plant is broadly developed spice in Bangladesh, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian nations just as our locale Damoh, India, additionally contributing perceived part towards the development of *Piper betle* (Family; *Piperaceae*) [7]. Herbal plant leaves show antimicrobial efficacy against human pathogenic microbes, investigated using dissolvable extraction and agar well diffusion process [8]. The oral disease causing bacteria are most lethal microorganisms they can cause deadly diseases like oral cancer and intestinal damages by secreting toxins.

## Materials and Methods

**Sample Collection:** Piper betle leaves were collected from Damoh district in India, washed, dried, and stored at 4ºC for further experimental use.

**Inoculum Preparation:** Piper betle leaves were dried at 40ºC, powdered, and soaked in solvents like methanol, ethanol, and aqueous layer for 72 hours. Using Whatman filter paper No. 1, the mixtures were filtered. Solvent stress was removed by soxhlet extraction, and the thicker compounds were separated after being dissolved in D2W and kept at 4oC.

**Test Bacteria Isolation & Purification:** Piper betle leaves are an edible mouthwash used to protect against pathogenic oral bacteria. Oral samples were used to isolate test bacteria using nutrient agar media plates. Grown colonies were inoculated using streak plate methods to achieve pure colonial bacteria.

**Enrichment & Identification:** Isolated bacterial colonies were tested for purification and differential characteristics, then transferred to enriched media for Gram staining. Light microscopes were used to identify and categorize colonies based on Gram staining characteristics.

**Antibacterial screening:** Antibacterial screening evaluates biologically vigorous solvent extraction derivatives using agar well diffusion method with slight amendment [9]. This research investigates antibacterial potency using methanol, ethanol fractions, and aqueous layers, with chloramphenicol as a positive control and pure solvents as reference. Sterilized distilled water as negative control and blank agar plate as control. Bacterial culture was inoculated, dissolved in 100µl water, and seeded on NAM agar plate using spread plate method. Wells (8mm diameter) were made using sterilized metal cork-borer and filled with test fractions. Media plates were incubated at 37ºC for 24-48 hours, with plates observed for zone of inhibition and diameters measured using Hi-media antibiotic zone mapping scale. Results were compared with a positive control and reference plate, and statistically analyzed for acceptance value and significance.

## Result and Discussion

**Sample processing and inoculum preparation:** Piper betle leaves from Damoh district in India were collected, processed, and stored for experimentation, with dried and powdered samples used. Three solvents were used to prepare an inoculum, and leaves were soaked, filtered, and coded for further experimentation. Lubis et al. (2020) used 300g dried Betle leaves dissolved in 96% ethanol and 10% DMSO [10].


**Fig 01:** *Piper betle* leaves, refined pulverize of dried leaves, Pulverize dissolved solvents

**Table No. 01: Sample collection, processing and inoculum preparation**

| S. N. | Sample | Sample Code | Local Name | Sampling Site |
|---|---|---|---|---|
| 1. | Piper Betle-01 | PBM | Bangla Paan | Damoh Rural |
| 2. | Piper Betle-02 | PBA | Bangla Paan | Damoh RDR |
| 3. | Piper Betle-03 | PBE | Bangla Paan | Damoh Rural |

Note: Random sampling performed during sample collection.

Test Bacterial Isolation, Purification and Identification: Oral spheres were used to isolate bacteria from teeth, saliva, and tongue. Gram staining identified four pathogenic bacteria: Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, and Streptococcus mutant. Isolated and identified bacteria were cultivated on enriched media namely EMB, MacConkey agar, Tryptic soy agar & MS agar media for differentiation (Fig.02 & Table 02). In accordance with this, Ermawati et al. (2021) stated that authenticated pure cultures of test bacteria, including Escherichia coli (ATCC 8739), Salmonella sp. (ATCC 14028), Staphylococcus aureus (ATCC 6538), and Pseudomonas aeruginosa (ATCC 9027), were obtained from ATCC. Bacterial suspension maintained, re-cultivated, and dilution method obtained active pure colonies [11].

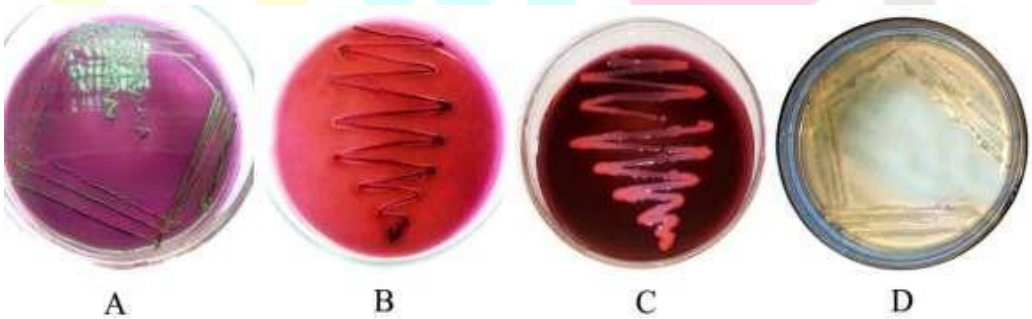



**Figure 02** Bacterial culture cultivated on enriched agar media plate. All purified culture identified using Gram staining. A- *Escherichia coli* (Gram Negative), B- *Staphylococcus aureus* (Gram Positive), C- *Enterobacter aerogenes* (Gram Negative) & D- *Streptococcus mutant* (Gram Positive).

**No. 02: Test Bacterial Isolation, Purification and Identification**

| S. N. | Source | Identified Bacteria | Shape | Observation | Gram's Nature |
|---|---|---|---|---|---|
| 1. | Saliva | *Escherichia coli* | Rod | Pale Pink/Reddish | Gram Negative |
| 2. | Tongue | *Staphylococcus aureus* | Round | Characteristic purple | Gram Positive |
| 3. | Tongue | *Enterobacter aerogenes* | Rod | Pale Pink/Reddish | Gram Negative |
| 4. | Teeth | *Streptococcus mutant* | Round | Characteristic purple | Gram Positive |

Note: All procedures performed in triplicate.

**Antibacterial screening by AWD method:** This study examined three solvent fractions and four oral pathogenic bacteria, including Gram-positive and Gram-negative strains, isolated from oral composites using swab method. Methanol fraction showed maximum zone of inhibition against all used test bacterial strains ranges from 25.00±1.00 mm to 23.33±1.52mm as compared to positive control. Methanol, Ethanol & Aqueous fraction showed significant activity against *Staphylococcus aureus.* Aqueous fraction showed considerable clear zone against *Streptococcus mutant* & *Escherichia coli* upto 18.33±0.57mm to 20.66±1.52mm. Moderate activity upto 17.66±0.57mm shown by all fractions against three test bacteria namely *Enterobacter aerogenes*, *Streptococcus mutant* & *Escherichia coli*. Where least activity 15.66±1.52 to 16.00±1.00mm observed against two strain *Escherichia coli* & *Enterobacter aerogenes* by aqueous fraction of *Piper betle* leaves. Positive control Chloramphenicol (10µg) used as positive control (Fig.03 & Table 03). Chakraborty et al. (2011) tested the antibacterial efficacy of Piper betle leaves against four human pathogenic bacterial strains using agar well diffusion method. They found broad spectrum activity against both Gram negative and Gram positive strains [12].

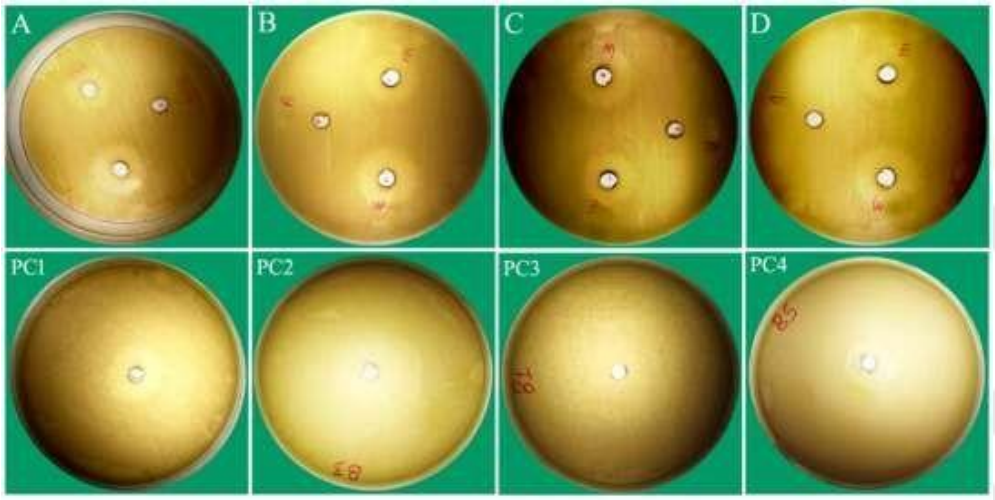


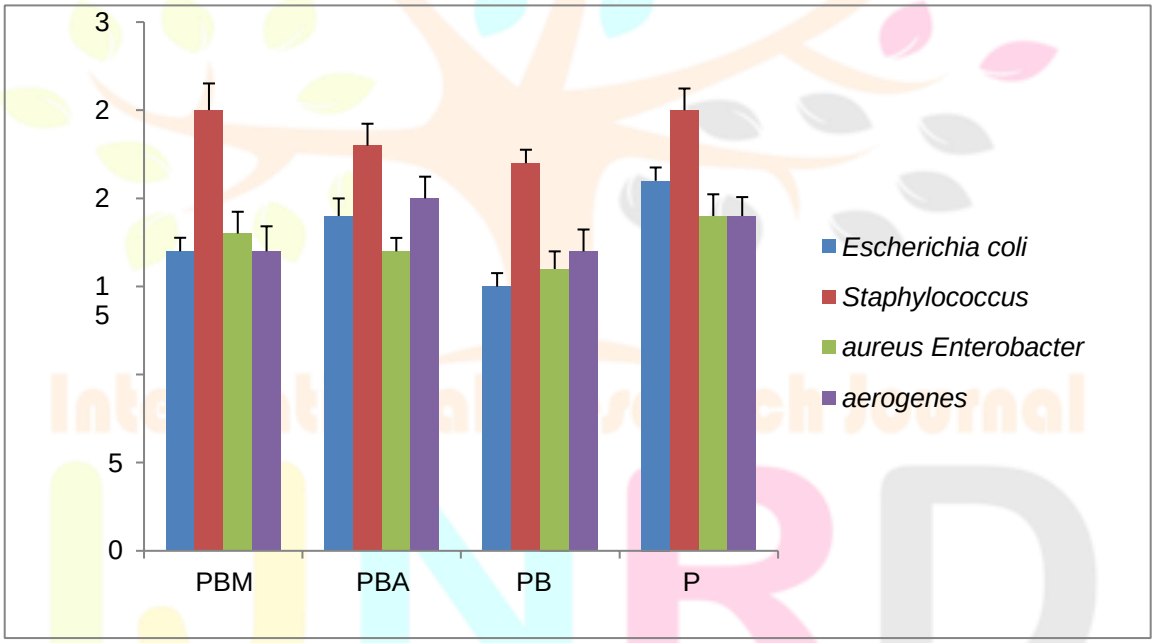

Fig 03: Plates showing broad spectrum antibacterial activity. Clear zone were measured using Hi-Media antibiotic zone measurement scale. A to D; Antibacterial screening of *Piper betle* leaves against A- *Escherichia coli* (Gram Negative), B- *Staphylococcus aureus* (Gram Positive), C- *Enterobacter aerogenes* (Gram Negative) & D- *Streptococcus mutant* (Gram Positive). PC1 to PC4 showing positive control for all plates (10µg Chloramphenicol).

**Table No. 03: Antibacterial Screening and statistical analysis**

| S.N. | Test Pathogenic Bacteria | Clear Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | PBM | PBA | PBE | PC |
| 1. | *Escherichia coli* | 17.333±0.577 | 19.333±2.309 | 15.666±1.527 | 21.000±2.000 |
| 2. | *Staphylococcus aureus* | 25.000±1.000 | 23.333±1.527 | 22.333±0.577 | 25.666±1.527 |
| 3. | *Enterobacter aerogenes* | 18.333±0.577 | 17.666±0.577 | 16.000±1.000 | 19.333±1.527 |
| 4. | *Streptococcus mutant* | 17.666±0.577 | 20.666±1.527 | 17.333±1.527 | 19.666±1.154 |

**Note:** Solvents were evaporated during Soxhlet procedure. Positive Control (PC)=Chloramphenicol. PBM= *Piper betle* Methanol, PBE= *Piper betle* Ethanol, PBA= *Piper betle* Aqueous. Values=Av±stdv.

**Statistical Analysis:** The outcome of all fractions and Positive controls (PCs) were analyzed to recognize potential standard error (SE) percentile followed by standard deviation (Stdv) along with persuasive fraction as well as relevant comparatives. Highest SE was ±1.519671 and minimum SE was ±0.759836. Standard error (SE) specified as percentage error bar in Graph 01.




Graph 01: Showing comparative antibacterial activity with standard error.

**Conclusion:** Herbal remedies treat diseases, and medicinal plants are used in pharmaceutical industries for bioactive medicines and antibiotics. In present study methanol & ethanol fraction of Piper betle leaves showed maximum zone of inhibition 25.00±1.00 mm and 22.33±0.57mm respectively against test oral pathogenic bacterial strains. Aqueous fraction also showed extensive activity 23.66±1.52mm against Streptococcus mutant & Escherichia coli. Research found significant inhibition zones of 25.00±1.00mm and 15.66±1.52mm compared to positive control, with statistical analysis using standared deviation and error.

This research pointed out a novel bio- remedy to kill pathogenic bacteria and to cure breathtaking diseases. *Piper betle* leaves are edible, herbal ecofriendly component that can be used as folk medicine as well as that can be boon

for bio-drug industry. Antibacterial potentialities of *Piper betle* leaves are remarkable to fight oral pathogenic bacteria as shown in this study.

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**Ethics Approval:** Applicable and Approved

**Consent to Participants:** Not Required

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