# Antibacterial Activity of *Piper betle* Leaf Extracts against Drug Resistant Bacteria of Social Relevance

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## Abstract

The emergence of drug resistance among bacterial pathogens is posing a threat to human lives. Among the safer methods of protection against drug resistant bacteria, medicinal plants are found to be socially relevant antimicrobial drug sources coming-up with appreciable results. At this juncture, a study has been aimed to test the antibacterial activity of a medicinal plant, *Piper betle* against drug resistant bacteria isolated from accidental wound samples of human subjects. The bacterial isolates, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were subjected to antibacterial assay using different solvent extracts of *Piper betle* leaves individually and in combination with the antibiotics using Kirby Bauer's disc diffusion assay. Among the different solvent extracts of *Piper betle* leaves tested, ethanol and aqueous extracts showed higher antibacterial activity against drug resistant bacterial isolates. The ethanolic leaf extracts of *Piper betle* showed a maximum zone of inhibition of 20.2 mm against *Escherichia coli* which is very close to the inhibition zone of 20.6 mm recorded in the standard antibiotic imipenem 10 µg/disc. The synergistic antibacterial activity studies showed that the ethanolic extracts of *Piper betle* in combination with imipenem recorded a higher zone of inhibition of 24.3 mm against *Pseudomonas aeruginosa* followed by 23.4 mm zone of inhibition in ethanolic extract + oxacillin against *Staphylococcus aureus*. The results reveal that there is a possibility of developing effective therapy for drug resistant bacteria by combining plant extracts and antibiotics.

***Keywords:*** Drug resistant bacteria, medicinal plants, antibiotics, *Piper betle*.

## Introduction

The drug resistance mechanism by pathogenic bacteria are reasonably exploding and causing serious health issues and economic loss. The source of origin of drug resistant bacteria are highly variable from vegetables, poultry products, drinking water, wound infections and so on (Nipa *et al*., 2011; Geidam *et al*., 2012; Adesoji *et al*., 2015; Ou *et al*., 2020). Wound infections are mainly caused by *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Enterococci* and majority of these bacterial isolates are found to be broad spectrum antibiotic resistant (Hirsch and Tam, 2010; Betts *et al*., 2016; Sharahi *et al*., 2020). Multi-drug resistant bacteria are commonly encountered in human samples. For instance, multi-drug *Pseudomonas aeruginosa* has been isolated from post surgical wounds (Al-Byti *et al*., 2020). Also, the most common Gram negative and Gram positive bacteria in skin and soft tissue infections is reported to be *Pseudomonas aeruginosa* and *Staphylococcus aeruginosa* respectively (Ukey *et al*., 2020).

There is a growing concern towards the use of synthetic antibiotics and the emergence of multi-drug resistance among the pathogenic microorganisms, especially bacteria. Hence, there is a need to search for alternative therapies from natural origin. Various treatment measures have been taken to control drug resistant bacterial isolates in wounds, which includes combination of antibiotics, nanomaterials, plant extracts, combination of plant derived products with commercial scale antibiotics. Medicinal plants are known to contain various active principles of therapeutic value and to possess biological activity against a number of diseases. These medicinal plants could be used against drug resistance bacteria. Various studies conducted world-wide show the great potential of plants as antimicrobial agents along with other biological activities, for example, taxol from *Taxus brevifolia* and gum-oleoresin from *Commiphora wightii* (Nicolaou *et al*., 1996; Sharma and Sharma, 1996). The extracts of medicinal plants alone and in combination of two or more plants have shown antibacterial activity against Gram positive and Gram negative bacteria (Karmegam *et al*., 2008). The *in vitro* antibacterial activity of traditional medicine, turmeric was found effective against extremely drug resistant bacteria synergistically with meropenem (Sharahi *et al*., 2020). The wound dressing materials coated with the extracts of *Calotropis gigantea, Eucalyptus globules*, and buds of *Syzygium aromaticum* showed enhanced results (Sampath Kumar *et al*., 2020).

The leaves of *Piper betle* (betel vine) is used in south Asia for traditional and religious ceremonies, and as medicine (Fazal *et al*., 2014). The leaf extract of *Piper betle* has been used to test for its antimicrobial activities against bacterial and fungal pathogens. Punareewattana and Aiemsaard (2016) reported that the antibacterial activity of *Piper betle* leaf extracts at 5% concentration was equivalent to that of 0.12% chlorhexidine against *Porphyromonas gingivalis*, a bacterium causing periodontitis. The betel vine leaf extracts in combination with conventional abtibiotics (50%:50%) showed bacteriostatic and synergistic activity against observed against *Staphylococcus aureus, Propionibacterium acnes, Staphylococcus epidermidis,* and *Streptococcus pyogenes* (Taukoorah *et al*., 2016). It has been proven that the betel leaves are effective against oral bacteria (Salam *et al*., 2014). Moreover, its activity against fungal pathogens and dermatophytic fungi is potentially effective (Trakranrungsie *et al*., 2008; Basak and Gupta, 2017). However, the studies pertaining to the effect of *Piper betle* leaf extract alone and combination with antibiotics against accidental wound isolated drug resistant bacteria are limited. The bacterial isolates in accidental wounds are socially relevant as they cause morbidity and mortality of human lives. Hence the present study has been carried out to find out the antibacterial effect of different solvent extracts of *Piper betle* leaves individually and in combination with antibiotics against drug resistant wound isolated bacteria,

*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*.

## Materials and methods

### Collection of *Piper betle* leaves

The plant, *Piper betle* for the present study was collected and identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983). After the conformity of the identification, the leaves were collected and transported to the laboratory. The leaves were then gently washed with running tap water and moisture was removed with blotting paper and air dried. The air-dried leaves were powdered using ball mill, packed in air-tight containers until use.

### Preparation of extracts

The Soxhlet extraction procedure was used for extracting leaves using the solvents, acetone, ethanol, petroleum ether, chloroform and aqueous extract as per the standard procedures. The leaves collected were dust removed, air-dried under shade, powdered and stored in polythene bags for further use. A known quantity of leaf powder (100 g) of each plant leaves was taken in Soxhlet apparatus using solvents individually. A greasy final material (crude extract) obtained for each plant was then transferred to screw cap tubes and stored under refrigerated condition till use.

### Antimicrobial assay

The drug resistant wound isolated bacteria, *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa* cultures maintained were used for the present study. The bacterial species were maintained by sub-culture method in nutrient agar slants. Kirby-Bauer's technique was used to test the sensitivity of selected test bacteria to *Piper betle* leaf extracts (Bauer *et al.*, 1966). Discs of 5 mm in diameter from a sheet of filter paper were punched out, placed in Petri dishes allowing a distance of 2-4 mm between each disc and sterilized in a hot air oven at 160ºC for 1 hour. After allowing the disc to cool, 20 µl (0.02 ml) of each test solution was added on to each disc and then the discs were dried at 37ºC in an incubator for one hour (Cheesbrough, 1984). For control set, the discs were added with distilled water (200 ml) containing 5ml ethanol + 2 drops of emulsifier at 20µl/disc. The agar plates were laid and incubated at 37ºC for 24 hours for each extract and for extract + antibiotic combinations (1:1). The zone of inhibition (ZI) was measured in mm and the results were recorded.

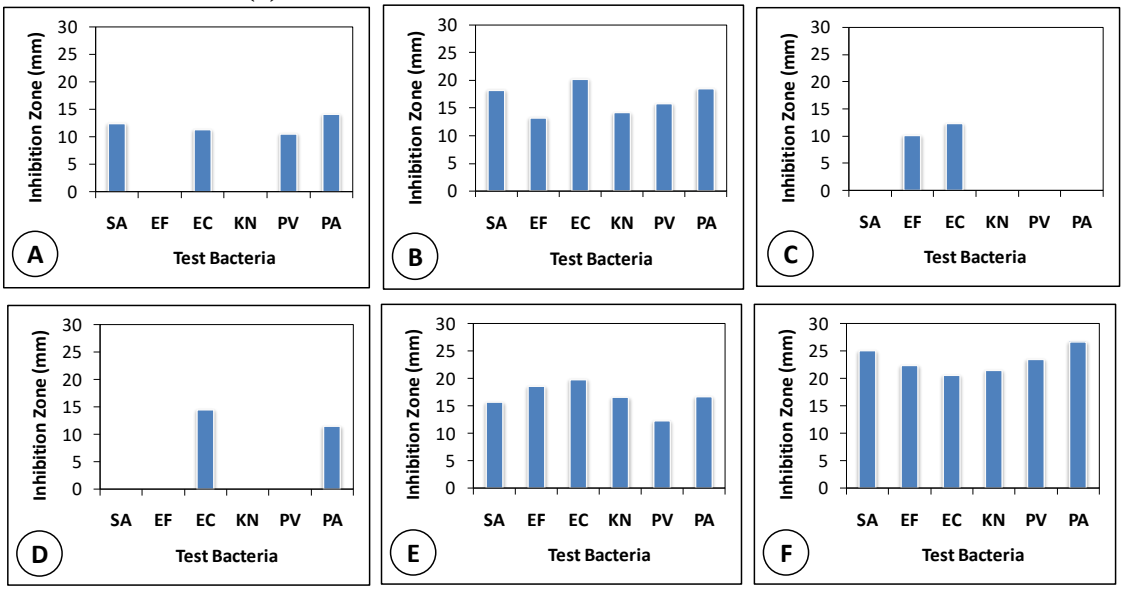
## Results and discussion

### Antimicrobial activity of different solvent extracts of *Piper betle* leaves

Among the different solvent extracts of *Piper betle* leaves tested, ethanol and aqueous extracts showed higher antibacterial activity against drug resistant bacterial isolates (Figure 1A – 1E). The ethanolic leaf extracts of *Piper betle* showed a maximum zone of inhibition of 20.2 mm against *Escherichia coli* which is very close to the inhibition zone of 20.6 mm recorded in the standard antibiotic imipenem 10 µg/disc followed by 18.5 mm and 18.2 mm zone of inhibitions

respectively against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Petroleum ether and chloroform extracts of *Piper betle* leaves did not show profound effect on test bacteria. The aqueous leaf extract of *Piper betle* recorded a maximum of 19.8 mm zone of inhibition against *Escherichia coli* followed by 18.6 mm against *Enterococcus faecalis*> 16.7 mm against *Pseudomonas aeruginosa*> 16.6 mm against *Klebsiella pneumoniae*> 15.7 mm against *Staphylococcus aureus*> 12.3 against *Proteus vulgaris*. Betel vine leaves are known to possess various biological activities, *viz.*, anti-depressant, antioxidant, antibacterial, antifungal, antiulcer and cardioprotective (Dwivedi and Tripathi, 2014). Caburian and Osi (2010) reported that the essential oil obtained from the leaves of *Piper betle* had significant antibacterial and antifungal activities. The findings of the present study have been well supported by previous studies carried out world-wide (Punareewattana and Aiemsaard, 2016; Taukoorah *et al*., 2016; Salam *et al*., 2014).

**Figure 1.** Antibacterial activity of *Piper betle* leaf extracts against drug resistant bacteria isolated from wound. *Staphylococcus aureus (SA), Enterococcus faecalis (EF), Proteus vulgaris (PV)* – Ofloxacin 5 µg/disc (AB); *Escherichia coli (EC), Klebsiella pneumoniae (KP), Pseudomonas aeruginosa (PA)* – Imipenem 10 µg/disc (AB). Leaf extract of *Piper betle* obtained using acetone (A), ethanol (B), petroleum ether (C), chloroform (D), aqueous (E) and standard antibiotic (F).



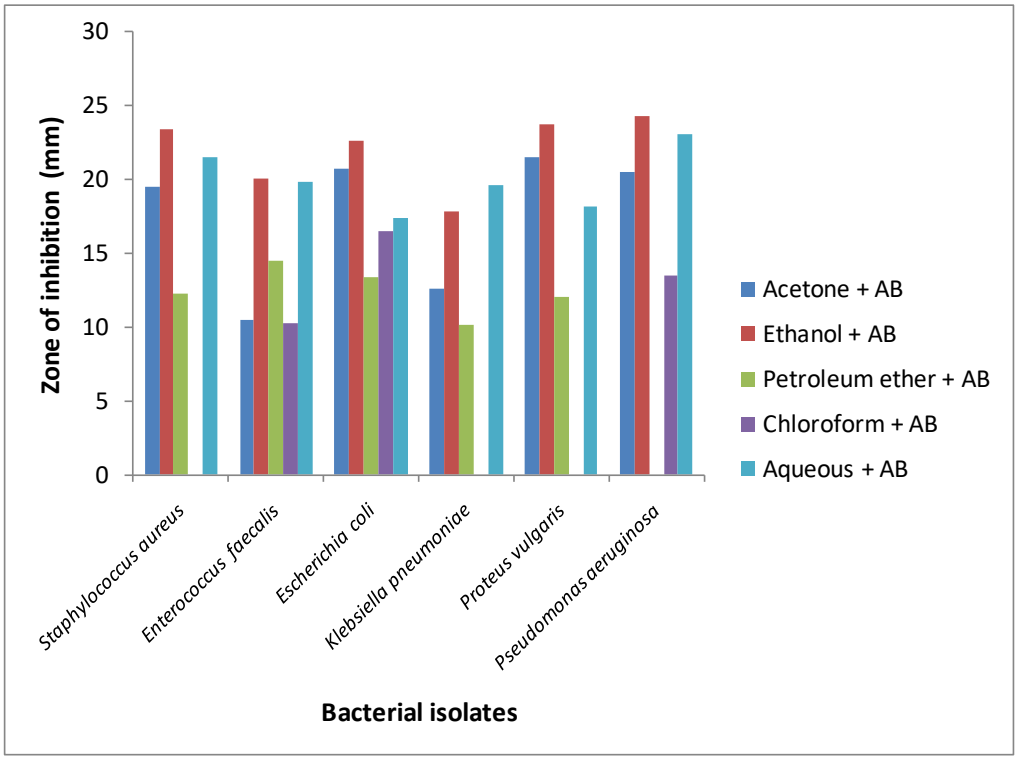

## Synergistic antibacterial activity of leaf extracts and antibiotics

The synergistic antibacterial activity of leaf extracts of *Piper betle* and *Piper sarmentosum* in combination with antibiotics showed higher activities. The ethanolic extracts of *Piper betle* in combination with imipenem recorded a higher zone of inhibition of 24.3 mm against *Pseudomonas aeruginosa* followed by 23.4 mm zone of inhibition in ethanolic extract + oxacillin against *Staphylococcus aureus* (Figure 2). The range of zone of inhibition for acetone extract in combination with antibiotics was 10.5 – 21.5 mm and it was 17.4 – 23.1 mm for aqueous extract + antibiotic combinations. The synergistic antimicrobial activity of petroleum ether extract + antibiotic and chloroform extract + antibiotic showed minimum activity only.

The plant extracts in combination with antibiotics have also been reported to possess enhanced antimicrobial activity. For instance, Moussaoui and Alaoui (2016) have studied the effect of essential oil extracted from medicinal plants in combination with antibiotics and concluded that the combinatorial effect of plant derived products and antibiotics may be useful against drug

resistant bacteria. Olayinka *et al*. (2009) reported that 60% of interactions of *Helichrysum pedunculatum* leaf extracts with antibiotics against wound infection associated bacteria is synergistic, hence these combinations could be relevant in combined therapy for persistent wound infections. The results of the current study findings fall in line with the synergistic effect of betel vine leaf extract with antibiotics (Taukoorah *et al*., 2016; Punareewattana and Aiemsaard, 2016). Conclusively, the combination of leaf extracts of *Piper betle* alone with antibiotics could be used to develop suitable formulations to combat drug resistant bacteria. Further studies on individual and combinatorial (extract of *Piper betle* + antibiotics) effect with reference to different ratios may provide more insights on specific antibacterial activity against drug resistant bacterial isolates from wound.

**Figure 2.** Synergistic antibacterial activity of *Piper betle* leaf extracts and antibiotics against drug resistant bacterial species isolated from wound. AB – Antibiotic; *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris* – Ofloxacin 5 µg/disc; *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* – Imipenem 10 µg/disc;

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