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EFFECT OF ETHANOLIC LEAVES EXTRACT OF *Piper nigrum & Piper betle* ON MULTI-DRUG RESISTANT MICROORGANISMS THAT AFFECT THE DIABETIC PATIENTS

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ABSTRACT

In Indian conventional medicine system, *Piper nigrum* and *Piper betle* are the commonly used medicinal plants for various therapeutic purposes such as treatment of antiulcer, antibacterial, antioxidant, anticancer etc., *Diabetics mellitus* is a disorder that occurs due to the improper functioning of pancreas in secreting insulin that leads to increase in blood glucose level which leads to various symptoms like frequent urination, loss of weight, obesity, damage to the cells, dizziness, low blood pressure and very frequent infection by pathogens such as *Escherchia coli, Staphylococcus mutans, Klebseilla pneumonia, Bacillus subtilis , Pseudomonas aeruginosa* and *Candida albicans.* In the present study aims to qualitative analysis the secondary metabolites from the ethanolic leaves extract of *Piper nigrum* and *Piper betle* and testing the ethanolic leaves extract against multidrug resistant microorganisms of diabetic foot ulcer. The results showed that the ethanolic leaves extract of *Piper nigrum* and *Piper betle* exhibited the effective antibacterial activity against the multidrug resistance organisms that affects the diabetic patients.

Keywords: *Piper nigrum*, *Piper betle,* Antibacterial Activity, Ethanolic extract and Phytochemical analysis

INTRODUCTION

Diabetics are a most dreadful disorder which is widely seen in the large population due to their unhealthier lifestyle and multi-factorial reasons. The patients are also more susceptible to developing infections due to weakening of their immune system, nerve damage and reduced blood flow to extremities thus they are highly prone to foot infections, yeast infections, urinary tract infections and surgical site infection. Some of these infections lead to mortality of patient because the microbes become drug resistant due to regular medication **[1-3]**. The medication that are given currently provided shows side effects one of the major impact of it is they get infected with the microbial infection that are resistant to drug which can be reduced by giving the patients with the extracts of the plant that contain the bioactive compounds **[15]**. In the present analysis the leaves of the two medicinal plants *Piper nigrum* and *Piper betle* were collected, powdered and extracted with the polar solvent ethanol. The effect of the extract was tested against three bacterial pathogens that predominantly infects the diabetic patients are *Bacillus* species, *Escherchia coli* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection of Plant Materials:

The leaves of the *Piper nigrum* and *Piper betle* were collected from the Kollimalai, Nammakal. It was identified and authenticated by Dr. S. John Britto, Director, Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. The sample was cleaned with distilled water thoroughly and shade dried. The dried leaves were powdered by electronic blender, and then extracted with the 30% ethanol solvent using Soxhlet apparatus at 40 °C. The extract was further dried with vacuum evaporator to obtain powdered form **[8-10]**.

Qualitative phytochemical analysis:

The ethanolic extract of *Piper nigrum* and *Piper betle* were dissolved in the distilled water and used for the further phytochemical analysis **[7, 14]**.

Test for alkaloid:

To a few drops of the both extracts Wagner's reagent was added and analysed for the formation of reddish brown precipitate

Test for Flavanoid:

Add a drop of ferric chloride to the few drops of both extracts and checked for the formation of blackish red precipitate

Test for phenol:

Equal volume of 5% glacial acetic acid and sodium nitrate added to the few

drops of the both extracts observed for the appearance of muddy niger brown colour

Test for steroid:

To a few drops of the *Piper nigrum* and *Piper betle* extract the chloroform and concentrated sulphuric acid were added and observed for the appearance of red colour

Antibacterial activity:

The multidrug resistant pathogens *Bacillus* species, *Escherchia coli* and *Staphylococcus aureus* were selected for the present study and done at SHIMPRA.

Agar well diffusion method:

The culture from the nutrient broth was swabbed on to the Muller Hinton Agar media which was autoclaved and poured in the petri-plate in sterile condition. The wells were created in the media with the sterile cork-borer and loaded with 40µl of the both plant extracts in two separate wells in each of the culture plate along with the standard antibiotic Gentamycin 20µl. The plates were then incubated at 37°C for 24 hours and the zone of inhibition was measured **[17]**.

RESULTS AND DISCUSSION

The qualitative phytochemical analysis of the ethanolic leaves extract of both *Piper nigrum* and *Piper betle* has showed the presence of Alkaloid, Flavanoid and steroid in whereas, the only phenol is present in the *Piper betle* and the *Piper nigrum* showed the absence of phenol **(Table 1)**. In previous research findings it was clearly mentioned that the alkaloids, flavonoids and steroids were possessed the greater antimicrobial activity against the clinical pathogens **[4, 5, 6]**.

The ethanolic extract in the 50µg concentration were loaded in the wells created in the swabbed culture plate of Muller Hinton agar plate with *Bacillus species, Escherchia coli* and *Staphylococcus aureus* has showed the maximum inhibitory effect against the multidrug resistant bacteria organisms as well as very low zone of inhibition by the *Piper nigrum* extract against *Staphylococcus aureus* and *Bacillus* species whereas lack of inhibition against *E.coli* when compared with *Piper betle* extract which produced maximum zone of inhibition against all the three organisms. Effect of the *Piper betle* against the pathogens was maximum against *Bacillus* sp followed in *Staphylococcus aureus* and *Escherchia coli*. The result **(Figure 1 and 2)** denotes that the presence of phenol compound in the betle leaves extract has increased the ability of the inhibition of the pathogens at the lowest concentration which is lacked by *Piper nigrum* **[11-15]**.

In the study of Lanchakon chanudom *et al*., (2014) **[16]** has taken crude extract from thirteen thai traditional plants and analysed the antimicrobial effect against norm *Staphylococcus aureus, Escherchia coli* and *Pseudomonas*

aeruginosa by disc diffusion method and broth dilution method. Among the thirteen plants the plants *Syzygium cumini* has showed the highest inhibition in the ethanol extract due to the availability of the phenolic content. This finding clearly supports the findings of our present research work. The results of the present research work was clearly showed that the ethanolic extract of *Piper nigrum* and *Piper betle* leaves exhibited a noteworthy antimicrobial activity against the multidrug resistant clinical pathogens that affects the diabetic patients.

Table 1: Results of qualitative phytochemical analysis

Name of the phytochemical	*Piper nigrum*	*Piper betle*
Alkaloid	+	+
Flavanoid	+	+
Phenol	-	+
Sterol	+	+

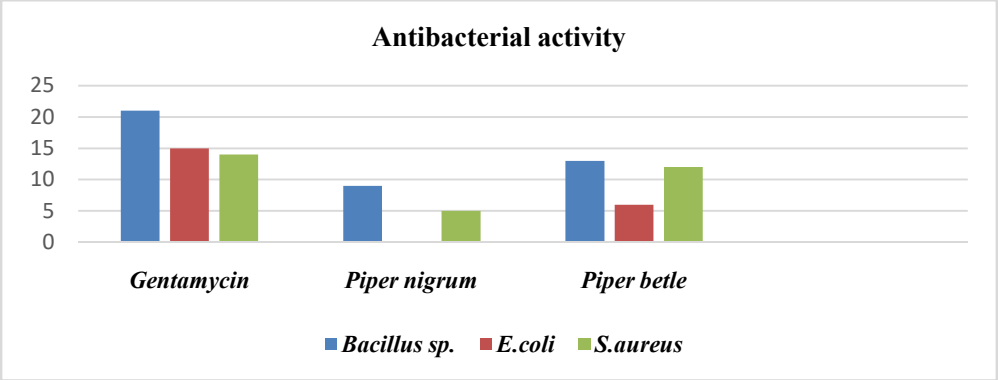

Figure 1: Antibacterial effect of *Piper nigrum* and *Piper betle*

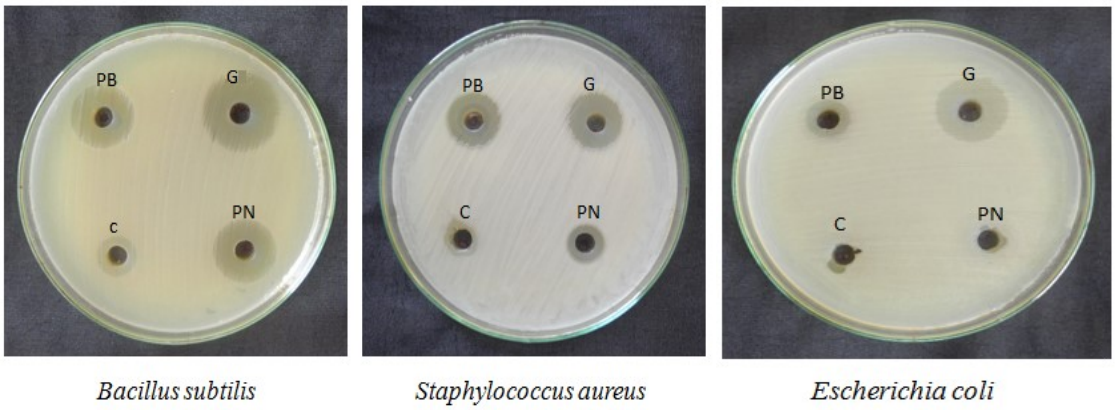

Figure 2: Antibacterial activity of *Piper nigrum* and *Piper betle*

CONCLUSION

It was clear that the ethanolic leaves extract of *Piper nigrum* and *Piper betle* leaves showed an appreciable antimicrobial activity against the multidrug resistant clinical pathogens that affects the diabetic patients. It was concluded that the *Piper nigrum* and *Piper betle* may be reservoir of potent phytochemicals that can utilized for the treatment of multidrug resistant

bacterial infections in the diabetic patients. The further research in these two plants may leads to the development of novel antimicrobial drug for the treatment of multidrug resistant bacterial infections in the diabetic patients.

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