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Evaluation of Antibacterial Properties of Extracts of *Piper betel* Leaf

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Abstract:

In the present study ethanolic and methanolic extracts of leaves of *Piper betel* were screened for antibacterial properties against pathogenic bacteria namely *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.* Both the extracts were effective against the used pathogens but methanolic extracts were found to be more effective in comparison to ethanolic extracts. Methanolic extracts showed maximum zone of inhibition of 25mm against *Staphylococcus aureus* followed by a zone of 17.5mm against *Pseudomonas aeruginosa* and 15mm against *Escherichia coli***.** Ethanolic extracts showed a maximum zone of 17mm against *Escherichia coli* followed by a zone of 16mm against *Staphylococcus aureus* and 14mm against *Pseudomonas aeruginosa.* MIC was found to be ranging between 0.0021mg/ml to 8.196 mg/ml in both the extract.

Key words: *Piper betel*, antibacterial properties, ethanolic extracts, methanolic extracts, agar well diffusion.

Introduction:

Large use of antibiotics has led to resistance against them in the pathogens, and the antibiotics have been found to be associated with side effects and addiction to the users [1] which are a major challenge for the researchers. It is the time to develop such drugs which are not associated with any side effects, and as they will be new for the pathogens there is no question of resistance. Best answer to this problem is the use of herbal medicines which have been used since time immemorial in Indian villages. The World Health Organization has adopted a major policy change wherein most developing nations have to make use of more traditional medical practices for primary health care [2].

Piper betel belonging to family *Piperaceae* is well known for its use as a mouth freshener since long, it is used post lunch and dinner in Indian families. However chewing *Piper betel* (Pan) in our society is not thought to be very good, but then also previous research on it have shown that it possess good antimicrobial properties. Lots of secondary metabolites have also been studied which have been found to be active against various pathogens.

The present study was designed to evaluate the antibacterial properties of the ethanol and methanol extracts of *Piper betel* leaves.

Material and method

Plant Material

Fresh and healthy leaves of *Piper betel* were collected from a Pan shop in Vibhuti Khand, Gomti Nagar, Lucknow and were brought to laboratory after proper identification. Leaves were washed with tap water followed by distilled water and dried, dried leaves were ground by the help of a grinder and the powder was used throughout the study.

Bacterial Strains and Culture Preparation

Three bacterial strains namely *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, collected from IMTECH, Chandigarh, were sub-cultured and used throughout the study.

Preparation of Plant Extracts

5g of powdered *Piper betel* leaves were soaked in 50 ml of 70% ethanol and 80% methanol. Kept in dark for 4 days so that secondary metabolites get dissolved. It was then filtered in weighed petriplates by the help of Whatman's filter paper No.1. After filtration the filtrate collected in weighed petriplates, was kept in oven at 50°C so that methanol and ethanol get evaporated. Dried metabolite was dissolved in double volume of 100mm Tris HCl, thus giving the final concentration of the extracted metabolite to 500mg/ml.

Antibacterial Susceptibility Assay

Antibacterial activity of the extracts was evaluated by the agar well diffusion method of Kirby Bauer with slight modifications wherein sterile NA plates were prepared and spreaded with 50 µl of pathogenic cultures against which the antibacterial activity is to be evaluated. Three wells of 8 mm diameter were bored using a sterile borer and the 1st well was loaded with 75 µl of standard antibiotic Tetracycline (50 µg/ml), 2nd with crude antimicrobial extract and the 3rd well with autoclaved distilled water, the plates were incubated at 37°C overnight. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period. All experiments were performed in triplicates.

Determination of Minimal Inhibitory Concentration (MIC):

MIC of the extracts (methanolic and ethanolic) was determined by broth dilution technique in which 12 test tubes with 3 ml of nutrient broth were autoclaved and cooled to room temperature. Two sets each containing 6 test-tube were made and the extract was serially diluted in both the tubes upto 10^{-5} dilution. One of the set was inoculated with the pathogens and incubated at 37°C for 24 hrs at 120rpm and the uninoculated set was preserved below 4°C and used as blank. After the incubation period the growth of pathogens in the test tubes was detected by reading the absorbance at 600nm. Concentration of the test tube in which growth of pathogen increased suddenly was called as MIC. All experiments were performed in triplicates.

Results:

Antibacterial Susceptibility Assay

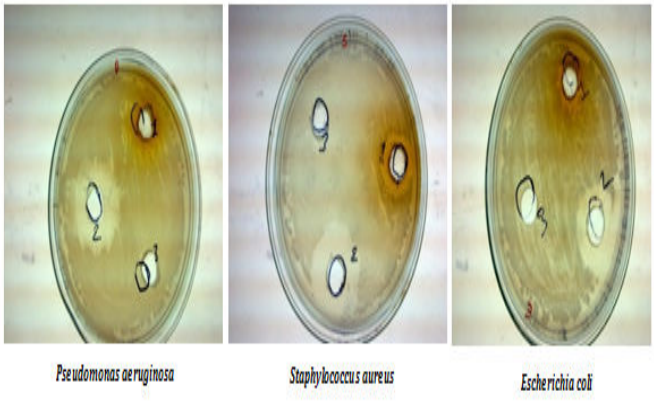
1.Antibacterial susceptibility assay of ethanolic extracts of Piper betel

Antibacterial susceptibility assay was performed for the ethanolic extract against various pathogens and the results of the same can be seen in ***Table 1*** **and** ***Figure 1*** below, it can be seen that maximum zone of inhibition was seen against *Escherichia coli* followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Individual, family and group therapy models were used for counseling.

Table 1: **Antimicrobial Susceptibility Assay of ethanolic extracts.**

TEST ORGANISM	ZONE OF INHIBITION BY TETRACYCLIN (in mm)	ZONE OF INHIBITION BY SAMPLE (in mm)
Pseudomonas aeruginosa	22 mm	14 mm
Staphylococcus aureus	22 mm	16 mm
Escherichia coli	19 mm	17 mm

Figure 1: **Antibacterial susceptibility assay of ethanolic extracts.**



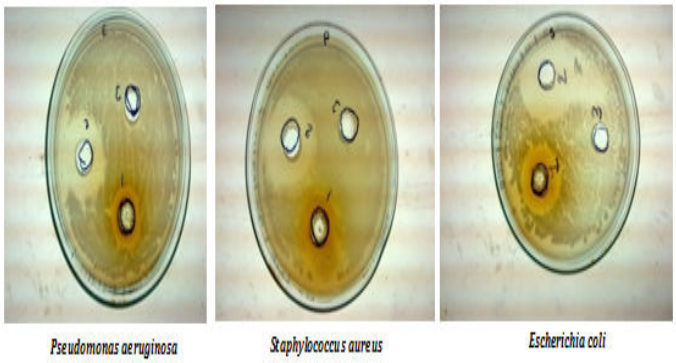
2.Antibacterial susceptibility assay of methanolic extracts of *piper betel*:

Antibacterial susceptibility assay was performed for the methanolic extract against various pathogens and the results of the same can be seen in ***Table 2*** **and** ***Figure 2*** below, it can be seen that maximum zone of inhibition was seen against *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* and *Escherichia coli.*

Table 2: Antibacterial susceptibility assay of methanolic extracts.

TEST ORGANISM	ZONE OF INHIBITION OF TETRACYCLIN (in mm)	ZONE OF INHIBITION OF SAMPLE (in mm)
Pseudomonas aeruginosa	25 mm	17.5 mm
Staphylococcus aureus	29.5 mm	25mm
Escherichia coli	25 mm	15 mm

Figure 2: **Antibacterial susceptibility assay of methanolic extracts.**



Determination of MIC

1.MIC of ethanolic extracts against pathogens:

MIC of ethanolic extracts was determined against all the three pathogens and the results can be seen in ***Table 3*** below.

Table 3: **MIC of ethanolic extracts against various pathogens.**

TEST TUBES	CONC. OF EXTRACT (in mg /ml)	O.D of *P. aeruginosa* at 600nm	O.D of *S. aureus* at 600nm	O.D of *E. coli* at 600nm
1	8.196	0.00	0.0	0.0
2	0.13	0.01	0.0	0.28
3	0.21×10^{-2}	0.12	0.0	0.45
4	0.34×10^{-4}	0.23	0.13	0.56
5	0.56×10^{-6}	0.38	0.45	1.79
6	0.91×10^{-8}	1.34	0.78	1.90

2.MIC of methanolic extracts against various pathogens:

MIC of methanolic extracts was determined against all the three pathogens and the results can be seen in ***Table 4.***

Table 4: **MIC of methanolic extracts against various pathogens.**

TEST TUBES	CONC. OF EXTRACT (in mg /ml)	O.D of *P. aeruginosa* at 600nm	O.D of *S. aureus* at 600nm	O.D of *E. coli* at 600nm
1	8.196	0.0	0.0	0.0
2	0.13	0.0	0.0	0.43
3	0.21×10^{-2}	0.01	0.21	0.64
4	0.34×10^{-4}	0.13	0.34	0.66

5	0.56 x 10^{-6}	0.34	0.54	0.76
6	0.91 x 10^{-8}	0.54	1.1	0.87

Discussion:

Herbal medicines are a valuable and readily available resource for primary health care and complementary care system. They can be the best alternative for the available antibiotics against which the pathogens are adapting resistance. *Piper betel* used as a mouth freshener can be a very good substitute for the available drugs after proper pharmacological investigation.

Plants extract were prepared from dried sample in this research work as has been reported earlier by [3]. Ethanolic and methanolic extract of *P. betel* leaves were taken for the antibacterial studies in the present research work, earlier [4-6] have reported antimicrobial properties of aqueous, ethanolic and methanolic extracts of *Piper betel*.

Agar well diffusion method was used here in order to determine the antibacterial properties of plant extracts against pathogens as has been performed earlier by [7].

Methanolic extracts showed maximum zone of inhibition of 25mm against *Staphylococcus aureus* followed by a zone of 17.5mm against *Pseudomonas aeruginosa* and 15mm against *Escherichia coli,* methanolic extracts have been reported to be effective by [6].

Ethanolic extracts showed a zone of 17mm against *E*scherichia *coli* followed by a zone of 16mm against *Staphylococcus aureus* and 14mm against *Pseudomonas aeruginosa,* [4] obtained a zone of 16mm against *Pseudomonas aeruginosa* and 13mm against *Staphylococcus aureus* by crude ethanolic extracts.

MIC was also performed to known the minimum inhibitory concentration of the extract by broth dilution method as experimented earlier by [8]. MIC was found to be ranging between 0.0021 mg/ml to 8.196 mg/ml in both the extract.

Conclusion:

Present investigation reveals the antibacterial nature of *Piper betel* leaves. Plant leaves were used for extraction of antimicrobial metabolites using ethanol and methanol. Out of the two extracts used methanolic extract was more effective. So Piper betel can be a tool for fighting against the pathogens which are gaining resistance to the drugs available in the market.

The future prospects of the present research work include isolation and purification of the therapeutic antimicrobials from the active extract and further pharmacological evaluation of the extracts and clinical trials.

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