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Original Article

Comparative analysis on effect of antibacterial activity of *Piper longum* leaf explants *in vivo* on *Escherichia coli* and *Bacillus subtilis*

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ABSTRACT

Antibacterial activity of *Piper longum* can protect from bacterial infection. *P. longum* prevents stomachache, diseases of spleen, respiratory tract, etc. Study on bacteria *Escherichia coli* and *Bacillus subtilis* give information about the extent of inhibitory effect of leaf explants of the plant. There was a comparison of effects in terms of percentage inhibition by preparing hot ethyl acetate extract of leaf explants, which was further calculated for both the bacterium.

Keywords: Antibacterial activity, *Bacillus subtilis*, *Escherichia coli*, *Piper longum*

Introduction

Microorganisms such as bacteria are causative agents of many diseases. Diseases caused by bacterial infection can be cured if antibacterial agents are used against them. Inhibitory effect of antibacterial property of leaf explants of *Piper longum* is beneficial to gather information necessary for treatment of diseases. Two bacteria studied in the present case are *Escherichia coli* and *Bacillus subtilis*. *E. coli* is Gram-negative bacteria and is causative agent of the diseases such as cholecystytis, bacteremia, cholangitis, and diarrhea. *B. subtilis* is Gram-positive bacteria, and it is non-pathogenic, it does not cause disease. In the present work, there is a comparison of the inhibitory effect of *P. longum* leaf explants on Gram-positive and Gram-negative bacteria. The percentage inhibition in each case was determined. Further, results of inhibition were better in *E. coli* as compared to *B. subtilis*.

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Objective

The objective of the study was to evaluate the antibacterial activity of leaf explants of *P. longum* on Gram-negative and Gram-positive bacteria, i.e., *E. coli* and *B. subtilis,* respectively, by preparing hot ethyl acetate extract of the leaf explants and to evaluate the inhibitory effects by calculating the percentage inhibition in each case.

Materials and Methods

P. longum plant was procured from botanical garden of National Research Institute of Basic Ayurvedic Sciences, Nehru Garden, Pune, Maharashtra, India. Bacterial culture, i.e., of *E. coli* and *B. subtilis* was procured from laboratory of Fergusson College, Pune, Maharashtra. Chemicals used were dimethyl sulfoxide (DMSO) and ampicillin.

Nutrient broth preparation

A media composition of tryptone - 10 g/l, sodium chloride - 10 g/l, yeast extract - 5 g/l, and distilled water - 1000 ml was prepared and adjusted to a pH of 7.2. Sample dilutions: Preparation of DMSO solution: (10 ml)-1 ml DMSO was added to 9 ml sterile nutrient broth in a sterile tube. 10 mg leaf extract was added to 1 ml DMSO stock solution.

Preparation of hot extracts

About 10 g leaf explant powder of *P. longum* was taken and mixed with 100 ml ethyl acetate it was then heated at 50°C and kept on

a shaker overnight, next day it was dried in rotavapor, then filtered using Whatman filter paper and was preserved at 4°C.

Serial dilutions

For leaf extract dilution was done in the ratio 1:1 (0.5 ml from stock solution was added to 0.5 ml DMSO), 1:2 (0.5 ml from stock solution was added to 1 ml DMSO), and 1:4 (0.5 ml from stock solution was added to 2 ml DMSO). These dilutions were prepared for a hot extract of leaf.

Preparation of bacterial cultures

About 100 ml of nutrient broth was prepared in two conical flasks, each were autoclaved, and then loopful of bacterial culture of *E. coli* and *B. subtilis* was inoculated in two flasks. It was then incubated on shaker for 2 h (O.D was 0.3 at 620 nm). Inhibition studies were done on ELISA plate.[2-6]

Hot extract of leaf and cell suspension of *E. coli* and *B. subtilis*

About 100 µl of cell suspension and 100 µl of each dilutions (hot extract of leaf) were pipetted in triplicate in the 1st row of plate. In case of positive control, 100 µl of cell suspension was added to 100 µl of ampicillin solution (1 ml sterile distilled water was mixed with 10 mg antibiotic and stored at 4°C) in the 2nd row of plate; it was pipetted in each well. In case of solvent control, 100 µl of DMSO was added to 100 µl of cell suspension in the 3rd row of plate. In case of negative control, 200 µl of cell suspension was added in each well in a 4th row. The plate was incubated at 37°C for 48 h. Absorbance was taken at 620 nm on ELISA reader and extent of inhibition was calculated.[1]

Results

In the case of *E. coli*, for 1:1 dilution of the plant, hot extract of leaf, the percentage inhibition was 4.32%, for 1:2 dilution it was 3.69%, for 1:4 dilution it was 2.25%. All the dilutions were taken in triplicates. On the other hand, in the case of *B. subtilis*, for 1:1 dilution of the plant hot extract of leaf the percentage inhibition was 5.18%, for 1:2 dilution it was 3.17%, for 1:4 dilution it was 2.17%. All dilutions were taken in triplicates in ELISA reader plate. Further, the results of positive control (ampicillin) for *E. coli* and hot extract of leaf explants used as a test sample was 74.47% whereas for *B. subtilis* the percentage inhibition for control was 71.23%.

Discussion

In the present work, in case of antibacterial activity, there was dose-dependent increase in percentage inhibition, ampicillin was taken as a positive control, this antibiotic has an antibacterial activity for bacteria used. It is used against *P. longum* as control to check the extent of antibacterial activity of its leaf explant. Previous studies which were carried on fruits of *P. longum* to check its antibacterial activity also showed dose-dependent increase in the percentage of inhibition.[2-4] The results of *E. coli* and *B. subtilis* showed less percentage of inhibition then their respective positive control. Moreover, the result of percentage inhibition for *E. coli* was better than that of *B. subtilis*. The optical densities for *E. coli* and *B. subtilis* are shown in Tables 1 and 2, respectively.

$$\text{Percentage of inhibition} = 100-\left(\frac{\text{Absorbance of test sample}}{\text{Absorbance of control sample}}\right)\times 100 \quad (1)$$

$$\text{Percentage of inhibition} = 100-\frac{\text{Mean of O.D. of the triplicates}}{\text{Mean of control O.D.of corresponding triplicates}}\times 100 \quad (2)$$

Conclusion

Results give information about the plant's medicinal value and its activity against two bacteria, namely, *E. coli* and *B. subtilis*. Comparative analysis between these two bacteria showed that leaf explants have a better inhibitory effect on *E. coli* as compared to *B. subtilis*. Hence, it

Table 1: Antibacterial property (*in vivo*) for hot extract of leaf and cell suspension of *E. coli*

Well No	1	2	3	4	5	6	7	8	9
E. coli+plant sample	0.911 (1:1)	0.912 (1:1)	0.921 (1:1)	0.908 (1:2)	0.904 (1:2)	0.901 (1:2)	0.911 (1:4)	0.909 (1:4)	0.905 (1:4)
E. coli+ampicillin positive control	0.239	0.240	0.241	0.242	0.243	0.245	0.241	0.245	0.239
DMSO+*E. coli* solvent control	0.343	0.337	0.323	0.341	0.341	0.343	0.347	0.337	0.332
E. coli negative control	0.951	0.956	0.961	0.939	0.941	0.937	0.937	0.938	0.936

E. coli: Escherichia coli, DMSO: Dimethyl sulfoxide

Table 2: Antibacterial property (*in vivo*) for hot extract of leaf and cell suspension of *B. subtilis*

Well No	1	2	3	4	5	6	7	8	9
E. coli+plant sample	0.912 (1:1)	0.867 (1:1)	0.567 (1:1)	0.956 (1:2)	0.808 (1:2)	0.678 (1:2)	0.909 (1:4)	0.832 (1:4)	0.689 (1:4)
E. coli+ampicillin positive control	0.238	0.240	0.241	0.244	0.246	0.234	0.212	0.234	0.267
DMSO+*E. coli* solvent control	0.341	0.343	0.345	0.340	0.341	0.356	0.311	0.321	0.346
E. coli negative control	0.897	0.698	0.961	0.939	0.941	0.937	0.937	0.938	0.936

B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, DMSO: Dimethyl sulfoxide

is necessary to carry study on the above bacterium to gain knowledge which is used to cure diseases caused by bacterial infection.

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