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. Piper longum prevents stomachache, Diseases of spleen, respiratory tract, etc.Study on bacteria Escherichia coli and Bacillus subtilis gives information about the extent of inhibitory effect of leaf explants of the plant. There was 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: Piper longum, antibacterial activity, Escherichia coli, Bacillus subtilis

Introduction

Micro-organisms 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 are beneficial to gather infor-mation necessary for treatment of diseases.Two bacteria studied in the present case are Escherichia coli and Bacil-lus subtilis. Escherichia coli is gram negative bacteria and is causative agent of the diseases like cholecystytis, bac-teremia, cholangitis, diarrhea, etc. Bacillus subtilis is gram positive bacteria and it is non-pathogenic, it does not causes disease. In the present work, there is compar-ison of inhibitory effect of Piper longum leaf explants on gram positive and gram negative bacteria.The percent-age inhibition in each case was determined. Further, results of inhibition were better in Escherichia coli as compared to Bacillus subtilis.

Objective

To evaluate antibacterial activity of leaf explants of *Piper longum* on gram negative and gram positive bacteria i.e, *Escherichia coli* and *Bacillus subtilis* respectively by preparing hot ethyl acetate extract of the leaf explants and to evaluate the inhibitory effects by calculating percentage inhibition in each case.

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Material and methods

Piper 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 *Escherichia coli* and *Bacillus subtilis* was procured from laboratory of fergusson college,Pune,Maharashtra. Chemicals used were Dimethyl Sulphoxide (DMSO) and Ampicillin.

Nutrient broth preparation

A media composition of Tryptone - 10 gm/l, Sodium chloride - 10 gm/l, Yeast extract- 5 gm/l and Distilled water – 1000 ml was prepared and adjusted to a pH of 7.2. Sample Dilutions: Preparation of Dimethyl Sulphoxide (DMSO) solution: (10 ml)- 1ml DMSO was added to 9 ml sterile nutrient broth in sterile tube. 10 mg leaf extract was added to 1ml DMSO stock solution.

Preparation of Hot Extracts

10 gm leaf explant powder of Piper longum was taken and mixed with 100 ml ethyl acetate it was then heated at 50°C and kept on shaker overnight, next day it was dried in rotavapour, 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 1ml DMSO), 1:4 (0.5 ml from stock solution was added to 2 ml DMSO). These Dilutions were prepared for hot extract of leaf.

Preparation of Bacterial cultures:

100 ml of nutrient broth was prepared in 2 conical flask, each were autoclaved and then loopful of bacterial culture of *Escherichia coli* and *Bacillus subtillis* was inoculated in two flasks. It was then incubated on shaker for 2 hrs (O.D was 0.3 at 620 nm).Inhibition studies was done on ELISA plate.

Hot extract of leaf and cell suspension of *Escherichia coli* and *Bacillus subtillis*

100 µl of cell suspension and 100 µl of each dilutions (hot extract of leaf) was pipetted in triplicate in 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 with10 mg antibiotic and stored at 4°C) in 2nd row of plate it was pipetted in each well . In case of solvent control 100µl of DMSO was added to 100 µl of cell suspension in 3rd row of plate,. In case of negative control 200 µl of cell suspension was added in each well in 4th row,. The Plate was incubated at 37°C for 48 hrs. Absorbance was taken at 620 nm on ELISA reader & extent of inhibition was calculated[1].

Results

In the case of *Escherichia 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 dilution was taken in triplicates.On the other hand in the case of *Bacillus subtillis,* 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 (ampicilin) for *Escherichia coli* and hot extract of leaf explants used as test sample was 74.47% whereas for *Bacillus subtillis* 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 positive control, this antibiotic has antibacterial activity for bacteria used. It is used against *Piper longum* as control to check the extent of antibacterial activity of its leaf explant.Previous studies which was carried on fruits of *Piper longum* to check its antibacterial activity also showed dose dependent increase in the percentage of inhibition[2,3,4].The results of *Escherichia coli* and *Bacillus subtilis* showed less percentage of inhibition then their respective positive control.Moreover the result of percentage inhibition for *Escherichia coli* was better than that of *Bacillus subtilis.*The optical densities for *Escherichia coli* and *Bacillus subtilis* are shown in table 1 and table 2 respectively.

Percentage of Inhibition =

$$100 - \left(\frac{\text{Absorbance of test sample}}{\text{Absorbance of Control Sample}}\right) X\ 100 \qquad (1)$$

Percentage of Inhibition =

$$100 - \frac{\text{Mean of O.D. of the triplicates}}{\text{Mean of control O.D of corresponding triplicates}} X\ 100 \qquad (2)$$

Conclusion

Results give information about the plant's medicinal value and its activity against two bacteria namely Escherichia coli and Bacillus subtillis. Comparative analysis between these two bacteria showed that leaf explants has better inhibitory effect on Escherichia coli as compared to Bacillus subtillis. So, it is necessary to carry study on the above bacterium to gain knowledge which is used to cure diseases caused by bacterial infection.

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

	1	2	3	4	5	6	7	8	9
A 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)
B E. coli+Ampicillin Positive control	0.239	0.240	0.241	0.242	0.243	0.245	0.241	0.245	0.239
C DMSO+ E.coli Solvent control	0.343	0.337	0.323	0.341	0.341	0.343	0.347	0.337	0.332
D E.coli Negative control	0.951	0.956	0.961	0.939	0.941	0.937	0.937	0.938	0.936

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

	1	2	3	4	5	6	7	8	9
A 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)
B E. coli+Ampicillin Positive control	0.238	0.240	0.241	0.244	0.246	0.234	0.212	0.234	0.267
C DMSO+ E.coli Solvent control	0.341	0.343	0.345	0.340	0.341	0.356	0.311	0.321	0.346
D E.coli Negative control	0.897	0.698	0.961	0.939	0.941	0.937	0.937	0.938	0.936

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