In vitro antibacterial activity of Emblica officinalis fruit extract by tube Dilution Method

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Abstract
The study was carried out to assess in vitro antibacterial activity of aqueous and organic extracts of Emblica officinalis fruits against four commonly encountered pathogenic strains of Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Pasteurella multocida. The present study revealed that there was significant reduction in the mean colony count of E. coli (293 ± 1.86 to 108.33 ± 0.67) and P. multocida (301.67 ± 2.03 to 139.67 ± 2.40) by the acetone extract, whereas there was significant reduction in the mean colony count of S. aureus (134.33 ± 2.40 to 256.33 ± 2.33) by the methanol extract and significant reduction in the mean colony count of K. pneumoniae (201.00 ± 2.31 to 267.33 ± 3.28) by the aqueous extract. The aqueous, acetone, ethyl acetate and methanol extracts were found to be effective against all the microorganisms under test where as chloroform extract was effective against E. coli and P. multocida but S. aureus and K. pneumoniae were found to be resistant against chloroform extract of E. officinalis. The outcome of the present investigation concludes that the E. officinalis fruit contain the antibacterial active ingredients.

Key Words: Emblica officinalis, antibacterial activity, tube dilution, bacterial resistance.

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diseases. The need for new therapeutics for infectious diseases has encouraged the drive to examine the nature and value of *E. officinalis* fruits. Therefore, an attempt was made to assess the antibacterial property of *E. officinalis* using tube dilution method against some common bacterial pathogens of both human and farm animals.

2. Material and Methods
2.1. Preparation of extract
The fruit powder of *E. officinalis* was procured from the authorized Ayurvedic pharmacy. The fine powder of fruit of *E. officinalis* was subjected for preparation of different extracts namely aqueous, acetone, chloroform, ethyl acetate and methanol. The extractability percentage was determined as per the method suggested by Rosenthaler [13].

2.2. Test organism
The test organisms selected in the present study based on their pattern of occurrence in the dairy animals and poultry. *S. aureus* and *E. coli* are the common pathogens causing mastitis, metritis, pyometra and other economically important infectious diseases in the dairy animals. Haemorrhagic septicemia caused by *P. multocida* leads to heavy economic losses in terms of reduced production and mortality especially in the buffalos. *K. pneumoniae* is the other important pathogen infecting the respiratory and reproductive system.

Pure cultures of *Escherichia coli* (MTCC, No.723) *Staphylococcus aureus*, (MTCC No.96), *Klebsiella pneumoniae* (MTCC No.106) and *Pasteurella multocida* (MTCC No.1161) were obtained from Institute of Microbial Technology, Chandigarh, India. The pathogenic bacterial culture was sub cultured and maintained on nutrient agar and in nutrient broth.

2.3. Preparation of extract impregnated disc
The sterile blank discs of filter paper were procured from M/s Hi Media Laboratories Ltd. Mumbai. These blank discs were separately impregnated with different extracts until the disc get fully saturated. The discs were weighed before and after impregnation of the extract. The amount of the *E. officinalis* extract actually got impregnated on to the disc was 19.79 ± 0.37 for methanol, 13.87 ± 0.29 for acetone, 12.26 ± 0.21 for aqueous, 9.91 ± 0.11 for ethyl acetate and 6.83 ± 0.47 for chloroform.

2.4. Determination of antibacterial activity
The antibacterial effectiveness of fruit extract of *E. officinalis* was determined by tube dilution method [14].

The test tube containing 3 ml of nutrient broth was added with a loop full of 24 hr old broth culture of each bacterium under test. The tubes were then incubated for 4 hrs. The bacterial dilutions were made ranging from $10^6$ to $10^0$. Two extract impregnated discs were placed in each tube and incubated at 37 °C for 24 hr. The broth in the tube was then plated on the nutrient agar taken in petri plates. After 24 hr of incubation at 37 °C, the bacterial growth in each petri plate was assessed by counting the bacterial colonies. Based on the observations the bacterial dilution of $10^6$ was selected for the final test.

For the final tube dilution test bacterial culture ($10^5$) taken in tubes were added with two extract impregnated discs and incubated at 37 °C. After overnight incubation the cultures (broth) were poured on nutrient agar taken in petri plates. The excess of broth was drained off after 3 minutes. After further incubation of 24 hr, the colonies were counted for each plate.

The data of this investigation were statistically analyzed by student t test and the results were represented as mean ± standard error [15].

3. Results
The mean colony count of *E. coli* without treatment was 293 ± 1.86. The mean colony count of *E. coli* with treatment of chloroform extract of *E. officinalis* fruit was 212 ± 1.15 followed by treatment of ethyl acetate (163 ± 3.79), aqueous (130.33 ± 0.88), methanol (12.67 ± 1.76) and acetone (108.33 ± 0.67) fruit extract of *E. officinalis*. The mean bacterial colony count of *S. aureus* without treatment was 256.33 ± 2.33. The mean bacterial colony count with methanol fruit extract was 134.33 ± 2.40 followed by acetone (148.33± 1.20), aqueous (160.67 ± 2.60), ethyl acetate (189.33 ± 1.20) and chloroform (227.00 ± 1.53) fruit extracts.

The mean colony count of *K. pneumoniae* without any treatment was 267.33 ± 3.28. The mean colony count by treatment of aqueous extract of *E. officinalis* (201.00 ± 2.31) was the lowest among all the extracts. The mean bacterial colony count by the treatment with methanol extract of *E. officinalis* fruit (210.33 ± 1.45) was statistically similar to the mean colony count by the treatment with Acetone fruit extract of *E. officinalis* (213.67 ± 3.18). The mean colony count by the treatment with ethyl acetate was 225.00 ± 2.31. The mean colony counts without treatment and with the chloroform extract (260.00 ± 3.18) were statistically similar to each other.

The mean colony count of *P. multocida* without treatment was 301.67 ± 2.03. The mean colony count of *P. multocida* by the treatment with Methanol fruit extract (142.33 ± 1.45) was statistically similar to the mean colony count of *P. multocida* by the treatment with Acetone extract of fruit of *E. officinalis* (139.67 ± 2.40). The mean colony count of *P. multocida* by the treatment with chloroform was highest among all the extract followed ethyl acetate, aqueous, methanol and acetone.

4. Discussion
The antibacterial activity of *E. officinalis* fruit extract was evaluated against four pathogenic bacteria belonging to both Gram positive and Gram negative group.

The acetone fruit extract has maximal activity against *E. coli* and *P. multocida*. The methanol extract has maximal antibacterial activity against *S. aureus* where as the aqueous extract have maximum antibacterial activity against *K. pneumoniae*. The results of the present investigation were in accordance with the reports of Saeed and Tariq [16], wherein, the *E. officinalis* have been found to be active against a range of bacteria including *Staphylococcus aureus*, *Haemolyticus*, *Saprobjecticus*, *Micrococcus varians*, *Bacillus subtilis* and also against *Candida albicans*. It was also apparent from the studies by the tube dilution that out of different extracts of *E. officinalis* the acetone fruit extract has the maximal antibacterial activity against *E.coli* than by other extract against other test organisms.
The antibacterial activity exhibited by the E. officinalis could be attributed to the presence of bioactive components namely flavonoids, phenols, saponins, tannins in the fruit extract [17]. Among these active principles the saponins are known to have potent bactericidal potency among the others. The fruit also contains tannins Emblicanin A and B, which are known to have potent antimicrobial activities [18].

Various investigators have reported that the fruits of E. officinalis have immune-modulatory activity [19, 20, 21]. The properties of immunomodulation and antibacterial potency of E. officinalis could be utilized synergistically for the treatment of infectious diseases in the immune-compromised individuals.

**5. Conclusion**

In the present investigation, the different solvent extracts of E. officinalis shown to have antibacterial activity against commonly encountered pathogens. The use of E. officinalis in the treatment of could be the safe, potent and cost effective way to treat infectious diseases of livestock, poultry and human.

**6. References**


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