

Evaluating Antibacterial Potential of Plant *Embelia Ribes* to Combat Resistant Microorganisms

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ABSTRACT

Background: Evidence from history has always shown the use of parts of plants or their extracts as folk medicines among people of different countries and cultural backgrounds. Herbs and plants supposedly provide us with over 100,000 secondary metabolites which can be used to treat several diseases and may act as antibacterial agents, anti-cancer agents, antimalarial agents, etc. The main reason to focus on herbalism in this modern era is to avoid the adverse effects of synthetic drugs and to provide treatment that is safe for both humans and the environment. We are witnessing an end of blind reliance on synthetic molecules as health safety concerns are arising. With increased antimicrobial resistance due to irrational use of antibiotics and impressive adaptations of pathogens to survive harsh environments and attacks of antibacterial agents, there is a need of looking for newer agents to treat infections. This world is now surrounded by an increasing number of pan-resistant bacteria (bacteria that are not susceptible to any clinically available antimicrobial agent). If not managed or countered on time we will soon be living in a post-antibiotic era. Some of the most problematic drug-resistant in the Gram-negative group of bacteria are multidrug-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* (also called ESKAPE organisms) are the cause of many ICU acquired infections in critically ill patients. These frighteningly increasing cases of antibiotic resistance are because of irrational and misuse of antibiotics. Though there is a need for new antibiotics it has been affected badly due to reduced economic motivation and impacted because of an increase in challenges in regulatory requirements as now very few new drugs are being developed by the pharmaceutical industry. Drug designers are actively looking at traditional medicine plants to explore new but safe molecules for antibiotics, but still, there are no commercially available antibiotics sourced from plants, in the market until now. Plant-based antimicrobial agents are unexploited reserves with impressive therapeutic potential and few side effects as compared to synthetic antimicrobials. *Embelia ribes* belongs to Myrsinaceae family, and its berries contain 4.33% of the embelin content. Embelin has proved to be natural antioxidant. It has also shown to down regulate viral replication in cell culture and exhibits inhibitory activity to extracellular virions. It has also exhibited bactericidal activity against Gram-positive and bacteriostatic activity against Gram-negative microorganisms.

Objective: The primary goal of this study was to evaluate the antibacterial activity of different concentrations of *Embelia ribes* extracted with two different solvent i.e. ethanol and hexane against drug resistant strains. Another objective of this study was to focus and compare the results of two antibacterial study methods including Disc Diffusion and Well Plate method.

Methodology: The antibacterial activity of the extracted drug was evaluated against drug resistant strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* and was compared with commercially available standard drugs to treat infections caused by these microbes. The selected strains of bacteria were first obtained from Essa Laboratory Karachi, Pakistan and then cultured and isolated in pure form in the university laboratory and were used to check its susceptibility with both ethanol and hexane extracts through Disc diffusion and well plate method in the concentrations of 250, 500, 750 and 1000 µg/ml each. Zone of

inhibition in all test plates were then measured carefully. The data was later analyzed by two-way ANOVA using SPSS followed by Tukey's test for posthoc analysis.

Result: Both ethanol and hexane extract demonstrated antibacterial activity against all three tested organisms. Embelin's antibacterial activity observed through disc diffusion method showed dose-dependent increase in zone of inhibition of hexane extracts against *P.aeruginosa* which is highly significant at 250 µg/ml, 500 µg/ml and 750µg/ml ($p<0.001$) as compared to ethanolic extract which only showed antibacterial activity at increased dose of 1000 µg/ml. Embelin's antibacterial activity against *P.aeruginosa* demonstrated more effectiveness of hexane extract than ethanolic extract in all test concentrations. When observed activity against *E.coli* through disc diffusion method, ethanolic extract showed dose dependent increase in zone of inhibition with all doses. However, two concentrations, 750 and 1000 µg/ml of ethanolic extract showed highly significant activity ($p<0.001$) when compared to hexane extract. Two concentrations i.e. 750 µg/ml and 1000 µg/ml of both extracts showed activity against *E.coli* through well plate method. A dose dependent increment in zone of inhibition out of all doses of ethanolic extract was observed against *Bacillus subtilis* with disc diffusion method however hexane extract is found to be effective at 750 µg/ml and 1000 µg/ml concentrations only. Ethanol extract showed effectiveness against organism with all concentrations except 250µg/ml through well plate method, against *Bacillus subtilis*.

Conclusion: This study has concluded that both ethanol and hexane extracts of *E.ribes* seeds have significant antimicrobial activity against drug-resistant microbes such as *P.aeruginosa*, *E.coli* and *Bacillus subtilis* when compared with standards drugs such as Piperacillin+Tazobactam, Ciprofloxacin and Ceftriaxone, statistically. It also demonstrated that out of two methods used for antibacterial activity test, Disc diffusion is more profound in giving self-explanatory results as compared to Well Plate method.