

Antibacterial Potential of *Emblica officinalis* Endophytes Against Multidrug-Resistant Avian Pathogenic *Escherichia coli* (APEC)Taha Mobeena^a, Rabia Tanvir^a, Muhammad Asad Ali^a and Wasim Shehzad^b^a*Institute of Microbiology (IOM), University of Veterinary and Animal Sciences (UVAS), Lahore 54000, Pakistan*^b*Institute of Biochemistry and Biotechnology (IBBT), University of Veterinary and Animal Sciences (UVAS), Lahore 54000, Pakistan***INTRODUCTION & AIM**

The poultry industry in Pakistan is a major source of animal protein and economic growth, but is severely impacted by colibacillosis, a disease caused by avian pathogenic *Escherichia coli* (APEC). The disease leads to high mortality in poultry, with symptoms such as respiratory distress and poor growth. Overuse of antibiotics has resulted in antibiotic-resistant *E. coli* strains, making treatment difficult and costly (Abbas and Zeeshan 2018). As a promising alternative, medicinal plants like *Emblica officinalis* (Indian gooseberry) have shown strong antibacterial effects against *E. coli*, attributed to their bioactive compounds, especially tannins (Tewari et al. 2019). Additionally, endophytes—microorganisms living within plants—produce antimicrobial substances that can combat multidrug-resistant bacteria. Harnessing these natural remedies may offer effective, sustainable solutions to manage colibacillosis and reduce reliance on antibiotics in poultry farming (Aslam et al. 2020).

METHOD**Collection of *Emblica officinalis* fruit and surface sterilization:**

Fresh fruits of *Emblica officinalis* were collected from markets in Lahore, identified, and washed thoroughly to remove debris.

The fruits were rinsed with distilled water, air-dried, and surface sterilized using a 2% sodium hypochlorite solution for 2–5 minutes, followed by three rinses in sterile distilled water to eliminate epiphytes (Kamil et al. 2023).

Isolation of endophytes:

Fruits were cut into 0.5 cm segments with a sterile scalpel and placed on nutrient agar (NA) plates, incubated at 37°C for 24 hours (Katoch et al. 2023). Alternatively, the fruits were ground into a slurry and streaked on nutrient agar plates. A control plate was established. Six isolates were obtained and maintained for further identification.

Preparation of *E. officinalis* endophytic extracts:

Each endophytic isolate was cultured in tryptic soy broth (TSB) at 37°C overnight. Equal volumes of ethyl acetate were added, and after phase separation, the organic phase was dried, dissolved in methanol, and stored at 4°C (Damavandi et al. 2023).

Antibacterial activity of *E. officinalis* endophytic extracts:

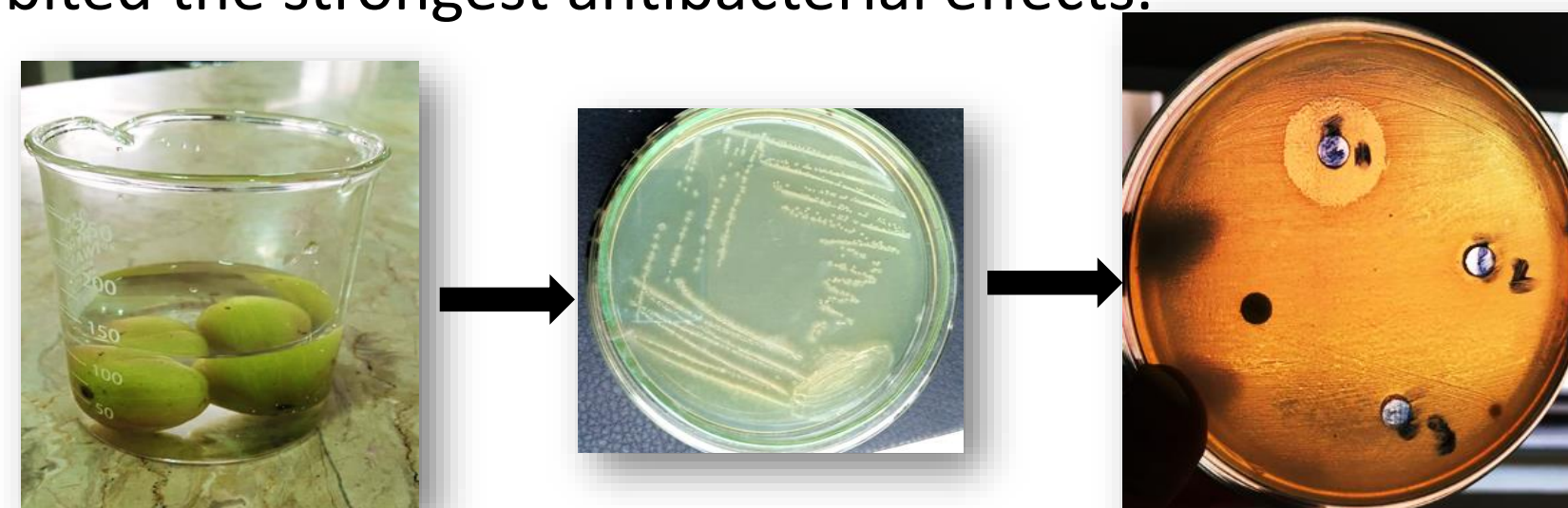
Antibacterial activity was assessed using the agar well diffusion method (Berdiyevich 2023) on Muller Hinton agar against *E. coli* isolates, with tetracycline as a control. Zones of inhibition were measured after incubation.

Partial purification by Thin Layer Chromatography (TLC) and staining:

TLC was performed to separate metabolites. Bands were visualized under UV light and sprayed with Kovac's reagent to identify peptide bands, which were then tested for antimicrobial activity (Alqurashi 2017).

RESULTS & DISCUSSION

A total of six endophytic bacterial isolates were successfully obtained and designated as EO1, EO2, EO3, EO4, EO5, and EO6. Gram staining revealed that EO1, EO2, EO4, and EO6 were Gram-positive rods, whereas EO3 and EO5 were Gram-negative rods, indicating a taxonomically diverse endophytic community. The antibacterial activity of each isolate was evaluated against three avian pathogenic *Escherichia coli* (APEC) strains using the agar well diffusion method. All extracts exhibited notable inhibitory zones, demonstrating the antibacterial potential. Against APEC strain 1, inhibition zones ranged from 14 to 20 mm, with EO5 showing the highest activity (20 mm). For strain 2, inhibition ranged between 10 and 28 mm, where EO6 demonstrated the greatest activity (28 mm). Similarly, for strain 3, inhibition zones ranged from 10 to 28 mm, with EO5 again displaying the largest inhibition zone (28 mm), followed closely by EO4 (26 mm). Overall, EO5 and EO6 consistently exhibited the strongest antibacterial effects.

**CONCLUSION**

Endophytes of *Emblica officinalis* fruit showed significant antibacterial activity against avian pathogenic *E. coli* so it can be useful against the poultry related infections. It can lead to an alternative solution against antibiotics in poultry farming.

FUTURE WORK / REFERENCES

- Abbas A, Zeeshan MJTEJoSS. 2018. Hurdles faced by small scale poultry industry. 1(9): 328-332.
- Alqurashi A. 2017. Identification and characterization of endophytic actinobacteria isolated from plants and sponges. Flinders University, School of Medicine.
- Aslam HB, Alarcon P, Yaqub T, Iqbal M, Häsler BJFivs. 2020. A value chain approach to characterize the chicken sub-sector in Pakistan. 7: 361.
- Berdiyevich DR. To determine the effectiveness of antibiotics in preventing chicken colibacteriosis based on experiments. European Journal of Agricultural and Rural Education. 2023;4(1):8-9.
- Damavandi, MS, Shojaei, H, Esfahani, BN. 2023. The anticancer and antibacterial potential of bioactive secondary metabolites derived From bacterial endophytes in association with *Artemisia absinthium*. 13(1):18473.
- Kamil MAIM, Abu-Bakar L, Reduan MFH, Kamaruzaman INA, Mahamud SNA, Azmi AFM, Rahman MSA, Nordin ML, Shaari R, Shean SCSJVIS. 2023. The use of medicinal plants in avian colibacillosis management: A Review. 21(2): 507-522.
- Katoch S, Singh R, Verma H, John JK, Jaiswal VJJoAR. 2023. Colibacillosis outbreak in one-week old Karaknath chicks at an organized poultry farm in Meerut, Uttar Pradesh. 13(2): 265-268.