Chapter-6

Summary and Conclusion

Endophytic microorganisms have an inexhaustible source of chemical compounds and can biosynthesize a wide variety of beneficial secondary metabolites. In search of the valuable characteristic of the endophytic bacteria, the present study explored eight bacterial strains showing different biological activities. So far, several bacteria on record have delivered highly potential drugs of pharmaceutical interest. Similarly, many medicinal plants have already been explored for drug invention. Nowadays, research on the endophytic bacteria residing in the medicinal plants gives a more evident vow of unveiling the signature drug for most dreaded diseases left without proper medicine. The current work aims to mark the beneficial aspects of endophytic bacteria in the leaves of two excellent medicinal plants, *M. citrifolia* and *M. pubescens*. Research on the endophytes reports that they utilize the chemical compound of the host for their metabolism and secrete it as extracellular metabolites. In vice versa, these endophytes are symbiotically related to the growth and development of the host plant. Based on this concept, the present work showcased the pharmacological activities of the endophytic bacteria.

In the present study, fifteen bacterial strains were isolated from the leaves of *Morinda* L. species, nine isolates from *M. citrifolia* and six from *M. pubescens*. The bacterial strain *Micrococcus yunnanensis* and *Bacillus marisflavi* were isolated from both the plants, indicating common metabolites or substrate in the host plants where they utilize. The metagenomic study conducted in the leaves of *M. pubescens* ensures the vast colonization of the endophytic bacterial community and diversity. Conclusively, four prominent phyla, Proteobacteria, Bacteriodetes Firmicutes, and Actinobacteria were identified to colonize the plant. Culture-independent methods applied in this work got a vague idea of endophytic bacteria prevailing in this plant, which was helpful while isolating the strains through a culture-dependent method.

Also, there are evidences in literature regarding metagenome analysis of bacterial community and diversity in *M. citrifolia* which made it useful in their isolation.

For the bioactivity study, culture-dependent isolation of bacteria on suitable culture media is inevitable. The present study selected eight strains from fifteen isolated endophytic bacteria based on distinct morphology and viable sub-culturing. All the chosen bacteria are pigmented and are strains of a different genus. Extracellular compounds extraction and qualitative GC-MS analysis of the isolates profiled various volatile organic compounds of bioactive principles from the selected strain. Also, the total phenolic content of the isolates were estimated, and the isolate *Bacillus vietnamensis* SMC has found to have more phenolic contents, that shows the probability of possessing anticancer property.

Nowadays, green chemistry-based biological methods such as bacterially induced nanoparticle synthesis or biomolecular template-based nanoparticle synthesis are effective in various fields due to their eco-friendliness. In addition, iron oxide nanoparticles contribute an outstanding category in medical fields. Moreover, bacterial-mediated iron oxide nanoparticle synthesis gets special applause in their diverse application. The study achieved in finding the endophytic bacterial strain-*Exiguobacterium aurantiacum* NMC1 capable of synthesizing iron oxide nanoparticles. The characterization indicates the synthesized nanoparticles have a surface plasmon resonance peak with maximum absorbance at 293.50 nm, characteristic of iron nanoparticles.

Furthermore, microscopy studies (SEM and TEM) specify that nanoparticles are spherical with diameters between 9.05 nm and 51.21 nm. Additionally, XRD analysis confirms the synthesized nanoparticles are γ-Fe_2O_3 structured, known as Maghemite. The dual advantage of maghemite acting as photocatalyst and magnetic nanomaterial makes it effective in degradation and removal of water pollutants. Therefore, the

present study has extended in preliminary assessment of applying the biogenic maghemite in heavy metal removal and dye decoloration were carried out. The result shows that these nanoparticles are very promising in environmental pollutants' bioremediation.

The pure colonies of the selected bacterial cultures were tested for their primary antimicrobial activity against human bacterial pathogens and plant fungal pathogens. As per the result, *Exiguobacterium aurantiacum* NMC1 showed strong antagonistic activities against the four tested pathogenic microbes, *Escherichia coli*, *Listeria* sp., *Klebsiella* sp., and *Streptococcus* sp. Thus, the result establishes that this endophytic bacterium has potent bactericidal activity whereas, the bacteria *Microbacterium kitamiense* REGI and *Micrococcus yunnanensis* STC have fungicidal activity against both *Aspergillus* sp. and *Sclerotium rolfsii*. At the same time, *M. yunnanensis* STC showed significant maximum growth inhibition of *S. rolfsii* and *Brevibacterium* sp. CVB represent maximum growth inhibition against *Aspergillus* sp.

In vitro environment was built using free radicals to create oxidative stress. DPPH free radicals, superoxide radicals, lipid peroxidation, and ferric ions could be scavenged, inhibited, or reduced by the isolates extract at different concentrations in a dose-dependent manner. The efficient DPPH radical scavenging property was analyzed in *Microbacterium* species, *M. kitamiense* (83.2%) *and M. paraoxydans* (82%). The maximum superoxide radical scavenging activity was exhibited by *Brevundimonas vesicularis* JAP (79.97%). Significant inhibition of lipid peroxidation was shown by *Brevibacterium* sp. CVB (70.17%). *Bacillus vietnamensis* SMC (76.27%) has a high ferric reducing capacity estimated in FRAP assay.

The extracts' in vitro short-term cytotoxicity study showed that *Brevundimonas vesicularis* JAP and *Bacillus vietnamensis* SMC are toxic to Dalton Lymphoma Ascites tested using the trypan blue method. In addition, long-term cytotoxicity

analyzed using MTT assay profiled viability of MCF-7 breast cancer cell lines by the extract at the predicted IC_{50} value for *Brevundimonas vesicularis* JAP (79.36 ±1.91 µg/mL) and *Bacillus vietnamensis* SMC (98.91 ±1.86 µg/mL). The toxicity study conducted in Swiss albino mice of these bacterial extracts suggested that they are safe for in vivo biological studies.

The study on the suppression of inflammation induced by carrageenan (acute) and formalin (chronic) in animal models suggests that the extracts of *Bacillus vietnamensis* SMC and *Brevundimonas vesicularis* JAP showed appreciable anti-inflammatory activity by significantly reducing the paw edema volume in mice. The findings establish that a high dosage (50 mg/kg b.wt.) of bacterial extracts showed a reduction in the mice's paw edema attenuated by carrageenan and formalin. The efficient anti-inflammatory activity conducted by bacterial extract could be explained by the potential antioxidant activity of the extracts. Thus, results opened that these bacterial extracts are helpful in the treatment of inflammation.

The present investigation demonstrates the significant antitumor activity of propylene glycol extract of NMT07 against ascites and solid tumors. The increase in lifespan in EAC-induced ascites tumor in mice was observed in standard and higher dose treated mice groups compared to control. In addition, administration of propylene glycol extract of *Bacillus vietnamensis* SMC (50 mg/kg b. wt) for ten consecutive days prior to tumor inoculation inhibited the tumor incidence by 78.97%, and whereas, 29.34% for lower dose (25 mg/kg b.wt) when compared to the standard drug (10 mg/kg b.wt) showing tumor volume suppression by 85.72% in DLA-induced solid tumor mice groups. Results also reveal that high dose extract is a more effective antitumor agent than the low dose in all the models compared to the standard drug. Nevertheless, its effect was found to be preventive rather than curative. Furthermore,

the antitumor activity of the sample extract is in a dose-dependent manner, with no signs of toxicity.

The significance of our research in using the extracellular secondary metabolites leftover in the bacterial culture supernatant can be justified in the following ways (1) to know the natural compounds that may be beneficial for humans (2) to identify the organisms producing these compounds for rational exploitation of them as they are the carrier of valuable substances (3) to produce nanoparticle from these useful compound efficiently and their screening in heavy metal removal and dye decoloration to benefit the environment (4) to ensure these compound are helpful to combat pathogenic microbes and (5) to explore these chemical substances in the anticancer study for the development of new potent drugs.

In the current study, the isolated bacterial strain from two medicinal plants belonging to the genus *Morinda* L. disclose maghemite nanoparticle synthesizing efficacy, antimicrobial potency, and antioxidant activity on scavenging different free radicals. Moreover, animal study such as anti-inflammatory and antitumor activity was reported. Thus, the present beneficial study on endophytic bacteria labeled the isolates' active compounds can be used in microbiology pharmaceuticals. Therefore, this work represents an essential step in understanding the endophytic bacterial compounds and their biological activity to benefit the medical field in further investigation.

In conclusion, the findings suggest that the selected eight endophytic bacteria species have important antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. Furthermore, the study focused on the use of the bacterial sample in nanoparticle production. The differences in these activities among the isolates can be attributed to their bioactive compounds. More studies on purification and structure elucidation of these bioactive compounds are needed to comprehend their beneficial effects better.