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Synergistic enhancement effect of the antibacterial activity of *Madhuca longifolia* (Mee) and zinc oxide nanocomposite

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Madhuca longifolia (Mee) is a deciduous tree that widely grows in Sri Lanka. Leaves of the *Madhuca longifolia* tree have proven antibacterial properties. Previous researchers have reported that Zinc oxide nanoparticles (ZnO NPs) also have notable antibacterial properties. But existing scientific literature do not provide any evidence on the possible synergistic enhancement of antibacterial effects of the *Madhuca longifolia* and ZnO nanocomposite compared to *Madhuca longifolia* and ZnO NPs alone. This research study mainly aims at addressing the above gap in scientific literature. Leaves of the *Madhuca longifolia* tree were the source of *Madhuca longifolia* powder. ZnO NPs were synthesized via the sol gel chemical synthesis method. Characterization of the ZnO NPs was carried out with UV-Visible spectroscopy, Fourier transform infrared spectroscopy and Scanning electron microscopy. *Madhuca longifolia* and ZnO nanocomposite were synthesized by incubating a mixture containing predetermined amounts of *Madhuca longifolia* leaf extract and ZnO NPs for a specified time period under specified conditions. Separate concentration series of *Madhuca longifolia*, ZnO NPs and *Madhuca longifolia* and ZnO nanocomposite were prepared as test solutions for the antibacterial assay. Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* and *Pseudomonas aeruginosa* were used as the test pathogens. All the studied test pathogens display statistically prominent zones of inhibition with *Madhuca longifolia* leaf extract and ZnO NPs compared to the positive control, suggesting that both *Madhuca longifolia* leaf extract and ZnO NPs have an appreciable antibacterial activity against both Gram positive and negative bacteria. When the *Madhuca longifolia* and ZnO nanocomposite is concerned, an increased inhibition is observed compared to the *Madhuca longifolia* leaf extract (p value = $5.85 \times 10^{-11} < 0.05$) or ZnO NPs (p value = $8.44 \times 10^{-5} < 0.05$) alone, against Gram negative bacteria. Fascinatingly, the above increment of antibacterial activity is higher at lower concentrations than at higher concentrations. This synergistic effect of enhancement can be attributed to the binding of ZnO NPs with the binding sites in the active phytochemicals of *Madhuca longifolia* such as flavonoids. The saturation of these binding sites with increased nanoparticle concentration may have decreased the increase in antibacterial properties. The antibacterial activity of *Madhuca longifolia* and ZnO nanocomposite against the Gram positive *Staphylococcus aureus*, is less than that of *Madhuca longifolia* leaf extract (p value = $0.49 > 0.05$) or ZnO NPs (p value = $0.11 > 0.05$) alone. The difference in the cell wall structure and composition between Gram positive and negative bacteria may have brought this change. *Madhuca longifolia* and ZnO nanocomposite could potentially be employed in the medical field to produce novel medications with lesser adverse effects as a promising countermeasure to the global threat of antibiotic resistance.

Keywords: *Madhuca longifolia* (Mee), ZnO nanoparticles, Nanocomposite, Antibacterial, Synergistic enhancement