ABSTRACT

Medicinal dyes have been used for centuries to treat ailments, however, research into their use as an antimicrobial has been lacking in recent years and most clinical work predates to 1945 and has now been overshadowed by antibiotics. Many antibiotics have become ineffective in treating the simplest of infections thereby sparking a new interest in combination therapy. This study aimed to explore potential synergistic effects of medicinal dyes and antimicrobials against pathogens (reference, resistant and clinical strains) responsible for skin infections.

To test the activity of the medicinal dyes and commercial antimicrobials, minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) assays were conducted against Gram-positive and Gram-negative bacteria, and yeasts. Combination studies were conducted in equal ratios (1:1) and the fractional inhibitory index (ΣFIC) was calculated. Varied ratio combinations were conducted on selected combinations and represented visually as isobolograms. Gentian violet demonstrated the strongest antimicrobial activity against all pathogens with a notably strong MBC value of 0.000031 µg/ml against *Staphylococcus epidermidis* (Clinical BA16), 0.01µg/ml against *Pseudomonas aeruginosa* (ATCC 27858 and DSM 46316) and *Escherichia coli* (ATCC 8379). It also demonstrated the strongest activity against *Candida albicans* with an MIC value of 0.00025 µg/ml.

Among the antibiotics, Mupirocin and Fusidic acid noted the strongest antimicrobial activity with an MIC/MBC of 0.04 µg/ml against *S. epidermidis* (Clinical BA16 and ATCC 51625), followed by Betadine® with an MIC/MBC of 1.56 µg/ml against *E. coli* ATCC 8379 and DSM 22314) and an MIC/MBC of 1.30 µg/ml against *C. albicans* (ATCC 10231). Individually, medicinal dyes noted stronger antimicrobial activity compared to conventional antimicrobials. Combination studies (1:1 ratio) noted 26% of dye-antibiotic combinations were synergistic against the Gram-positive strains, 15% against the Gram-negative strains and 14% against the yeasts. In the varied ratio studies, the Mercurochrome: Betadine® combination noted synergy among all the *Staphylococcus aureus* strains with the 1:1 ΣFIC values ranging from 0.05 to 0.48. Fuchsine with Gentamycin noted the most synergistic ΣFIC value of 0.001 against *P. aeruginosa* (DSM 46316)

strain. Methylene blue with Ketoconazole demonstrated the best synergistic ΣFIC value of 0.01 among the yeasts.

The brine shrimp lethality assay was then undertaken to determine the toxicity of the medicinal dyes, commercial antimicrobials, and selected combinations (used in varied ratio studies). Four dyes (Iodine tincture, Gentian violet, Malachite green and Mercurochrome) demonstrated high toxicity at 24 and 48 hrs. All four dyes demonstrated a percentage mortality of 100% at 48 hrs. The majority of the commercial antibiotics were non-toxic at both 24 and 48 hrs. Combination studies (1:1) on selected (50%) synergistic combinations observed a decrease in toxicity. The combination of Gentian violet with Gentamycin noted a fifteen-fold decrease in toxicity – the most synergistic combination. Malachite green in combination with Neomycin noted the second highest decrease in toxicity.

The selectivity index (SI) of the medicinal dyes, commercial antimicrobials and selected combinations were calculated based on the brine shrimp lethality assay together with the MIC. A low SI (<10.00) was observed amongst the majority of the dyes tested. The lowest SI was demonstrated by Iodine tincture with a SI value of 0.0001 against all *S. aureus* strains, Gram-negative strains, and yeasts. The commercial antimicrobials noted higher SI values in comparison with the medicinal dyes. The highest SI value observed was 12500.00 against *S. epidermidis* (ATCC 12228) when calculated against Fusidic acid. Gentamycin demonstrated high SI values among majority of the Gram-negative strains. All three antifungals demonstrated high SI ratios among *C. albicans* (ATCC 10231 and A100). For the combinations tested, Gentian violet with Gentamycin demonstrated the highest SI of 977.50 against the *E. coli* (DSM 22314) strain.

Time-kill studies were conducted on the combinations with the highest safe SI value and lowest safe SI value: Gentian violet with Gentamycin and Malachite green with Neomycin respectively. Both medicinal dyes demonstrated bacteriostatic activity over a period of 24 hrs. When combined, Malachite green with Neomycin demonstrated bactericidal activity.

This study has demonstrated that selected dye: antimicrobial combinations (20.46%) potentially act synergistically, however, when the antimicrobial activity and toxicity of these combinations

have been investigated, two combinations demonstrate more promise in comparison to the others: Gentian violet with Gentamycin and Malachite green with Neomycin. These combinations have the potential for further clinical studies as a future treatment for skin infections. This study also highlights the importance of further evaluation of the antimicrobial properties of medicinal dyes in combination with commercial antimicrobials in order to determine viable options to treat skin infections.