ABSTRACT

Development of microbial resistance is one of the major threats to the survival of mankind. To overcome drug resistance, numerous therapies have been used like new drug discovery, modification of antibiotics and administration of combination of two or more antibiotics. The most common and best practiced remedy to treat microbial infections observed to be the traditional medicinal plants and their combinations with antibiotics. In the present study, 72 medicinal plants extracts were screened to get synergistic activity in combination with erythromycin. Among all the medicinal plant, Colebrookea oppositifolia which was selected to analyze the effectiveness of the combination with erythromycin and amoxyclave, as it has shown synergistic activity in all plant parts in combination with erythromycin and amoxyclave. The class I synergism (increase in the zone of inhibition) was exhibited by petroleum ether extract of C. oppositifolia (leaves) with the increase in the zone of inhibition of amoxyclave from 6±0.2mm to 8±0.2mm and petroleum ether extract (inflorescence) from 6±0.2mm to 10±0.2mm respectively, whereas the antibacterial activity of leaves with zone of inhibition was 6±0.1mm and inflorescence extracts was 8±0.1mm respectively against S. aureus. The methanol leaf extract of C. oppositifolia showed class I synergism in combination with erythromycin and amoxyclave against S. aureus. Interestingly the methanolic leaf extract has not shown any antibacterial effect against S. aureus, but enhances the zone of inhibition of erythromycin from 3±0.1mm to 9±0.2 mm and amoxyclave from 5±0.2mm to 9±0.2mm. Class II synergism (making drug bactericidal) was exhibited by methanol leaf extract against S. aureus. The methanolic leaf extract of C. oppositifolia and erythromycin, both were bacteriostatic against S. aureus. The synergistic effect was more pronounced in leaves as compare to inflorescence and bark, as leaf extract was not showing any antibacterial activity alone against S. aureus. The sequential fractionation of the methanolic extract using solvent extraction (n- butanol, ethyl acetate) showed the class I synergism when combined with amoxyclave and erythromycin. The solvent fraction of ethyl acetate has increased the zone of inhibition of erythromycin from 4±0.3mm to 11±0.2mm and in the case of amoxyclave from 8±0.1 to 12±0.1 against S. aureus. The n- butanol fraction increases the zone of inhibition of erythromycin from 4±0.1mm to 12±0.2mm, whereas the zone of inhibition of amoxyclave was increased from 8±0.1mm to 13±0.1mm by against S. aureus. The ethyl acetate and n- butanol fractions were not observed to be antibacterial alone.
Though the chloroform fraction showed antimicrobial activity of zone size of 8±0.2 but has not shown synergism/ additive effect in combination against *S. aureus*. The solvent fractions have not shown class I synergism against *E. coli*. Class II synergism was shown by n- butanol and ethyl acetate fraction, in which both of these fractions enhances the potency of erythromycin by making it bactericidal. The volatile oils (inflorescence and leaves) extracted from *C. oppositifolia* have not shown antibacterial activity but surprisingly enhances the antibacterial activity of erythromycin and amoxyclave. The class I synergism was observed by volatile oil (leaves and inflorescence) of *C. oppositifolia* against different strains of bacteria. The synergistic activity was observed in volatile oil of leaves of *C. oppositifolia* against *S. aureus, K. pneumonia, S. pyogenes, S. enterica* and *S. epidermidis* with erythromycin and amoxyclave. The volatile oil (leaves) was only antibacterial for *S. enterica*. The volatile oil of (inflorescence) of *C. oppositifolia* was observed to be synergistic with amoxyclave and erythromycin against *S. pyogenes, S. enterica* and *S. epidermidis*. The volatile oil (inflorescence) has shown zone of inhibition of 2±0.4mm against *S. pyogenes*. Class II synergism was observed by leaf oil of *C. oppositifolia* against *S. aureus*, inflorescence oil of same plant against *S. aureus* and *S. sonnet*, making erythromycin bactericidal in combination. Further analysis of volatile oil by GCMS showed α- pinene (23.85), caryophyllene (11.312), δ- carene (13.642) as the major terpenoids. Antimicrobial assays were performed for δ- carene, α- pinene, caryophyllene and combination therapy of these compounds with erythromycin and amoxyclave. No synergistic effect was observed in these combinations against *E. coli* and *S. aureus*. From the study of UV- visible spectrometry, FTIR, HPLC and NMR, no complex formation was observed in the combination mixture of terpenoids and antibiotics.

**Keywords:** Antibacterial, medicinal plants, synergism.