SUMMARY AND CONCLUSION

The aim of present study is to explore the diversity of plants of Solan and Shimla to screen the extracts which potentiate the antimicrobial activity of traditional antibiotics, for which microbes are developing resistance. To achieve this aim we have screened 71 medicinal plants of this region to get best synergistic combinations with erythromycin and amoxyclave against *S. aureus* and *E. coli*. 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±0.2 mm to 8±0.2mm and petroleum ether extract (inflorescence) from 6±0.2mm to 10±0.2mm respectively, whereas the antibacterial activity of leaf extract with zone of inhibition was 6±0.1mm and inflorescence extracts was 8±0.1mm was observed against *S. aureus*. Interestingly the methanolic leaf extract did not show any antibacterial effect against *S. aureus*.

The bioguided fractionation of the methanolic extract using polar/ non polar solvent extraction (n- butanol, ethyl acetate and chloroform) showed the ethyl acetate and n- butanol fraction 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±0.3 mm to 11±0.2 mm, whereas amoxyclave from 8±0.1 to 12±0.1 mm against *S. aureus*. Similarly the n- butanol fraction increases the zone of inhibition of erythromycin from 4±0.1 mm to 12±0.2 mm, whereas the zone of inhibition of amoxyclave was increased from 8±0.1mm to 13±0.1mm against *S. aureus*. The ethyl acetate and n- butanol fractions did not antibacterial activity when tested alone. Though the chloroform fraction showed antimicrobial activity with a zone of inhibition of inhibition of 8±0.2 mm but did not show synergism effect in combination against *S. aureus*.

As the Lamiaceae family is very well known for the essential and volatile oils, so volatile oil was extracted from the leaves and inflorescence parts of *C. oppositifolia*, to find potent synergism with erythromycin and amoxyclave. 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 synergistic activity was observed in volatile oil of leaves of *C. oppositifolia* against *S. aureus* (5±0.2 mm), *K. pneumonia*
(12±0.1 mm), *S. pyrogenes* (7±0.3 mm), *S. enterica* (12±0.2 mm) and *S. epidermidis* (8±0.1 mm) with erythromycin and *S. aureus* (9±0.3 mm), *K. pneumonia* (6±0.2 mm), *S. pyrogenes* (12±0.1 mm), *S. enterica* (10±0.1 mm) and *S. epidermidis* (13±0.1 mm). The volatile oil (leaves) was only antibacterial for *S. enterica* (2±0.3 mm). The volatile oil of (inflorescence) of *C. oppositifolia* was observed to be synergistic with amoxyclav against *S. aureus* (9±0.3 mm), *K. pneumoniae* (6±0.1 mm), *S. pyrogenes* (10±0.1 mm), *S. enterica* (7±0.2 mm), *S. sonnei* (8±0.2 mm), *S. epidermidis* (12±0.1 mm) and with erythromycin against *S. pyrogenes* (13±0.1 mm), *S. enterica* (12±0.1 mm), *S. epidermidis* (10±0.1 mm). The volatile oil (inflorescence) has shown zone of inhibition of 2±0.4 mm against *S. pyrogenes*. Class II synergism was observed by leaf oil of *C. oppositifolia* against *S. aureus*, inflorescence oil against *S. aureus* and *S. sonnei*, by 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 with erythromycin and amoxyclav. No increase in the zone of inhibition was observed in combinations with terpenoids (δ-carene, α-pinene, caryophyllene) and antibiotics (erythromycin and amoxyclav) against *E. coli* and *S. aureus*. Whereas in combination of these terpenoids with each other, the synergistic activity was observed in α-pinene and δ-carene with zone of inhibition of 12±0.5 mm against *E. coli* and 13±0.4 mm against *S. aureus*. From the study of UV-visible spectrometry, TLC, FTIR, HPLC, docking and NMR the interaction was observed but no complex formation occurred in the combination mixture of terpenoids and antibiotics.

Thus it is concluded that to control a particular disease in vitro experiments should be carried out with various antibiotics, their combination as well as antibiotics and plant extracts, so that a right combination could be formulate to cure the patient for early and safe recovery from a specific disease. All the combinations do not produce synergistic effect and therefore extensive screening for the combinations are required to be tested. For this the medicinally rich biodiversity of Himachal Pradesh can be explored to search more potent combinations of medicinal plant extracts and traditional antibiotics, to put step forward to formulate more drugs based upon the herbal combination with less toxicity and low dosage.