6. CONCLUSION

Medicinal plants have been used for centuries as a source of medicine. The global importance of medicinal plants can be illustrated by the numerous conventional drugs that have been derived from plants and are currently used in clinical practice. Some examples of these drugs are quinine, atropine, opioids and taxol. Interest in medicinal plant research has escalated, with the aim of identifying alternative antimicrobial therapies to overcome resistance. There is, however, general consensus amongst the various studies, that plant derived antimicrobials possess a lower potency than conventional antimicrobials. Furthermore, antimicrobial resistance against conventional antimicrobials has been on the rise and has become a major public health concern. This has propelled research in the direction of combination therapies for enhanced efficacy. Many researchers have studied antimicrobial interactions between natural products, as well as combinations of natural products with conventional therapies.

In the present investigation, the antimicrobial activities of the hexane, chloroform, ethyl acetate and methanol extracts of the leaves and seeds of four Cassia species viz., Cassia alata, Cassia auriculata, Cassia fistula and Cassia tora collected from various places of Tamilnadu, India were studied. Hexane, chloroform, ethyl acetate and methanol extracts were prepared from the Cassia species and were screened against Gram-positive bacteria viz., Bacillus subtilis, B. pumilus, Micrococcus luteus and Staphylococcus aureus, Gram-negative bacteria
viz., *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* and fungi *viz., Aspergillus flavus, A. fumigatus, A. niger* and 10 isolates of Methicillin Resistant *Staphylococcus aureus*. Antibacterial and antifungal activities were studied by disc diffusion method. Minimum inhibitory concentrations (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were also determined.

In the present study, the hexane, chloroform, ethyl acetate and methanol extracts of four species of *Cassia* had possessed differential antibacterial and antifungal activities with mean zones of inhibition from 6.3 to 25.6 mm. The minimum inhibitory concentration and minimum bactericidal/fungicidal concentrations were from 62.5 to 500 µg/mL and 125 to 1000 µg/mL respectively. Among the four species of *Cassia*, the ethyl acetate extract of leaves of *Cassia tora* showed the highest antibacterial and antifungal activity for ethyl acetate extract (mean zone of inhibition-25.6 mm). In addition, methanolic extracts showed the highest antibacterial and antifungal activity than that of chloroform and hexane extracts. The control drug, Ciprofloxacin (10 µg/disc) was used for standard bacterial strains and recorded mean zones of inhibition ranged from 27.0 to 33.5 mm and Ketoconazole (5 µg/disc) was utilized for standard fungal strains and produced mean zones of inhibition ranged from 9.3 to 11.1 mm. Methicillin (5 µg/disc) was used as positive control for MRSA strains and produced mean zones of inhibition from 7.00 to 9.10 mm. The blind control, 10 per cent DMSO did not inhibit the growth of tested strains.
In the present study, among the hexane, chloroform, ethyl acetate and methanol extracts tested against bacterial and fungal strains, the lowest MIC (62.5 µg/mL) and MBC (125 µg/mL) values were recorded with ethyl acetate extract against *Staphylococcus aureus*.

The separation of active principle was carried out from the ethyl acetate extract of leaves of *Cassia tora* (due to the highest zone of inhibition) using column chromatography packed with silica gel and different fractions were obtained. The active fraction was analysed for the identification of the compound using spectral studies namely, FT-IR, GC-MS, $^1$H NMR and $^{13}$C NMR analyses. Based on the spectral studies, the fraction was identified as hexadecanoic acid ethyl ester.

The different concentrations of hexadecanoic acid ethyl ester were tested against the bacterial and fungal strains and the results showed antimicrobial activity against all the bacterial and fungal strains tested. The highest mean zone of inhibition (27.1 ± 0.28 mm), the lowest MIC (7.81 µg/mL) and the lowest MBC (15.62 µg/mL) were recorded against *Staphylococcus aureus*.

Further, chemical and pharmacological investigations are recommended to the pharmaceutical industry for commercial applications. The pharmacological investigation with hexadecanoic acid ethyl ester would be helpful to develop new type of antimicrobial agent from natural source.