CHAPTER-4

Summary and

Conclusion
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Plants are rich source of secondary metabolites. Invention and use of medicinal plants are date back to prehistory, in India it is since vedic period onwards and for the rest of the world, the system of identifying and using these medicinal plants have been found in 3000 BC itself. In the earlier period man used crude methods for healing and keeping good health and extracts of herbal plants were obtained in crude form which could not exhibited the results much to the satisfaction. Hence the researchers progressed as the civilization developed and present invention of GC-MS solved many more problems to identify the basic principles of plant and its volatile oils. Likewise several techniques were developed to identify and isolating the microorganisms.

In the present doctoral research work a systematic approach was made to find the efficacy of the tested volatile oils against microorganisms including skin pathogens and *Aedes aegypti*.

Clevenger apparatus was used to obtain volatile oils of the tested plants. GC-MS analysis by adopting kovat’s procedure was applied to know the components of the volatile oils. Microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and among fungi *Candida albicans* and *Trichophyton mentagrophytes* were isolated and identified by following NCCLS, method.

Efficacies of volatile oils against microorganisms were analyzed by antimicrobial susceptibility studies. Subsequently larvae and adult mosquito of *Aedes aegypti* were breded in lab condition in cement tubs attached with net and organic manure was given as a diet and placed in cement tubs and thus kept ready for larvicidal, lethal effects and repellency effects of plants volatile oil. The breeding of mosquitoes was done at

Any class research work needs some objectives followed by findings. Hence the objectives are placed in the chapter of introduction of the present work for fruitful results. The main aim of the present work is to determine the efficacies of extracts or volatile oils of selected medicinal plants against microorganisms and Aedes aegypti. The other objectives such as isolation and identification of fungal skin pathogens, to determine the lethal or repellent effects of volatile oils, are correlated to the above main objective.

Based on the results obtained, the following findings are presented in three categories for clarity and unambiguity.

They are:

I. Findings observed in extracts of selected plants.

II. Findings observed on efficacy of selected plants volatile oils as a controlling agent against microorganisms.

III. Finally the findings on larvicidal, adulticidal and repellency properties of the plants volatile oil against Aedes aegypti.

Extracts of the selected medicinal plants were obtained by soxhlet method and three types of extracts of each plant namely methanol, hexane and water extracts were obtained and studied the antimicrobial properties of these extracts which was stated as under:

Methanol extract when compared with the hexane extract, methanol extract showed high efficacy on water borne followed by food borne and fungi. Extracts of methanol and water when compared with hexane extract, methanol and water extracts were more effective on tested microorganisms as the secondary metabolites of the compounds of these extracts released immediately, which contributed their action to control the
microorganisms. Of all the methanol and water extracts, *L. aspera* plant had high efficacy on water borne and food borne pathogens but less active on fungal strains. The water extracts of *A. aspera* plant showed a good results and the extract activity against microorganisms were found in the order of water borne followed by fungi and food borne pathogens. Hexane extract of this plant also shows promising results. But in case of *Leucas aspera* water extract, the microorganisms such as *Proteus vulgaris* followed by *E. coli, Pencillium rubrum* and *Chaetomium globosum* were all sensitive. Further the water and methanol extracts of this plant had shown promising effect on fungi. Though the extracts of these plants were comparatively less active with their plant volatile oils, the extract solutions were useful for their percentage yield that placed these extracts for commercial production where as volatile oils were highly effective but their percentage yield was very less.

Volatile oils possessed rich components that had good effect on microorganisms. GC-MS analysis of these tested plant volatile oils revealed the presence of sulphur or sulfonates or sulfides components and carvacrol, carvone, myrcene, iso-propyl phenol, and menthol components in *Leucas aspera*; . All these components contributed promising antimicrobial properties. In addition, active compounds were found in each plant volatile oils that were as follows: *Leucas aspera*: caryophyllene, alloaromadendren, cyclopropeazulene; in *A.aspera* plant: 2(1H)-naphthalenone, uvidin; were all noticed as active components of volatile oils of plants.

The volatile oils of *A.aspera* seed was studied and found that the volatile oil is highly effective as controlling agent on microorganisms due to the presence of thymoquinone component in its volatile oils. The microorganisms that were sensitive to volatile oils
were in the order of *Haemophilus influenza* followed by *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio cholerae*, *Pencillium rubrum* and *Neisseria gonorrhoeae*. Among the pathogens *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*, *Candida albicans* and *Trichophyton mentagrophytes* which revealed that the volatile oils had good effect on gram positive followed by gram negative, yeast and fungi. The findings revealed that the efficacy of the plant volatile oils was more active on bacteria rather than fungi. The plant volatile oils inhibited the cell wall of the bacteria as per the silver plate inhibition test, tested in *Staphylococcus aureus* skin pathogen.

The present study on *Leucas aspera* volatile oils was the first report of its kind. This leaf volatile oils showed good contribution as controlling agent both on bacteria and fungi. Good results were found on mycosis infection. *Candida albicans* followed by *Trichophyton mentagrophytes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were all sensitive to volatile oils. In other words the volatile oils of the plants leaf had good efficacy on yeast followed by dermatophytes, gram negative and gram-positive bacteria. Overall results showed that the volatile oils were more effective on fungi than bacteria.

Further the overall findings as stated in the above paras showed that all these tested plants volatile oils had high efficacy on microorganisms when compared with the standards. Comparing with the standard, the volatile oils were more effective on microorganisms relating to influenza, diarrhea, cholera, enteric pathogens and also on skin bacterial and mycosis pathogens. Another finding for effective volatile oils against microorganisms was comparing the relation between ZOI and MIC to arrive the formula $\text{ZOI} \propto \frac{1}{\text{MIC}}$ with some minor exceptions depending on strain-to-strain differences.
Further studies on protein profile of *Staphylococcus aureus* were analyzed by gel electrophoresis. The observations showed that many exoproteins bands in the gel were due to oxidative stress in the organism and the stress was due the volatile oil effect.

In the present doctoral research work the tested plants volatile oil were further studied to know the efficacy of the volatile oils as vector control agents and to assess the potentials of the components as alternative to chemical substances. For assessing the efficacy of the plants volatile oil as controlling agent on *Aedes aegypti*, the statistical procedure adopted by Finney was followed and accordingly 50% lethality of larvae, adult and 50% effective dose concentrations for repellency effect were analyzed. The 50% lethal dose and 50% effective dose of the plant volatile oils against *Aedes aegypti*.

Further it was found that at low concentration the volatile oils had the ability to repel the mosquito but for adulticidal the assay needs much more concentration of the volatile oils. The presence of citronellol, eugenol, geranaiol, linalool, thymol and estragole components in the volatile oils of the plants contributed the larvicidal, adulticidal and repellent properties.

Another finding was that the repellency depended on both concentration of the volatile oils and time after application as the volatile oils evaporated from skin with time. Similarly toxicity of volatile oils for larvicidal was both time as well as time dependant. The lowest concentration of *Leucas aspera* volatile oils enhanced the moulting of the larvae whereas at higher concentrations the volatile oils killed the larvae.

Further the plants volatile oil of lethal concentrations when applied on skin, no symptoms of irritation or hot sensation or rashes were observed.
All the above findings suggested that the volatile oils of the tested plants showed good efficacy as controlling agents of mosquito that could be useful in search of newer, more selective and biodegradable natural compounds.