CHAPTER 6

CONCLUSION

6. Conclusion

- During survey 40 different herbs and spices were collected and authenticated.
- Among them six popular herbs (*Mentha piperita, Trigonella foenum-graecum, Coriandrum sativum, Murraya koenigii, Glinus oppositifolius* and *Foeniculum vulgare*) and six aromatic spices (*Illicium verum, Myristica fragrans, Ceiba pentandra, Capsicum annuum, Parmelia perlata* and *Dregea volubilis*) hadbeen selected for the evaluation of biological activities.
- Plant samples were cleaned, dried and powdered and extracted with hot water and methanol, followed by lyophilization to obtain lyophilized aqueous and methanolic extracts respectively.
- Preliminary phytochemical screening to detect the presence or absence of some significant phytochemicals viz. phenols, flavonoids, tannins, alkaloids, cardiac glycosides, saponins, terpenes, steroid, etc. were performed according to standard protocols. Phytochemical analysis revealed that phenol, flavonoid, reducing sugar, free amino acids, tannins were detected in all plants tested; anthraquinone was absent in *I. verum, C. pentandra* and *P. perlata*; triterpenoid was not detected in *C. sativum, T. foenum-graecum* and *M. fragrans*;cardiac glycosides were detected in all samples except *T. foenum-graecum, I. verum* and *C. pentandra*;alkaloid was detected in all the test plants; saponin was absent in *C. sativum*; steriod was absent in *C. annuum*; phlobatannin was present only in *P. perlata* and *D. volubilis* and cardenolide was presen only in *D. volubilis.*
- Samples were evaluated for the total soluble sugar content, reducing sugar content, soluble protein content and total lipid content, total phenol, flavonoid and flavonol content along withvitamin C and E.*G. oppositifolius and I. verum had h*ighest amount of total phenol, total flavonoid, total flavonol, total sugar and vitamin C.Highest amount of reducing sugar and total and vitamin E was present in *G. oppositifolius and I. verum.* Protein content was found to be highest in *M. koenigii,* lowest in *G. oppositifolius.*

- Quantity of different plant pigments like total chlorophyll content and total carotenoid content was estimated and found highest in *G.oppositifolius* for both the pigments. Carotenoid content was lowest in *M. piperita*.

- Extraction of the samples with methanol revealed higher yield than hot aqueous which may be due to the higher solubility potential of phytochemicals in methanol. Amongst the samples, *P. perlata* showed the highest yield.

- In vitro antioxidant activity in terms of free radical scavenging activity revealed that both the sample extracts of *I. verum* and *O. oppositifolius* had highest scavenging activity. In all the cases, gradual rise in the activity with the increase in the concentration was observed but it was insignifcantly different to each other in case of majority of herb extracts.

- Different biological activities reveals that methanolic extract was most active in compared to aqueous extract. Hence, methanol extract was thus selected for further studies on antimicrobial activity, anti-quorum sensing activity and antidiabetic activity.

- Antimicrobial activity of methanolic extracts of different herbs and spices against both Gram positive bacteria and Gram negative bacteria by disc-agar diffusion method. Revealed that among the herb extracts, *M. piperita* and *T. foenum-graecum* were found inhibiting *B. cereus* and *B. pumilus.* The MID values of *M. piperita* and *T. foenum-graecum* against *B. cereus* were 8.5 and 3.5 mg extract $disc^{-1}$ respectively, whereas MID values of the same plant extracts against *B. pumilus* was found to be 5.5 and 7.5 mg extract $disc^{-1}$ respectively. Rest of the herb samples (*C. sativum*, *M. koenigii*, *G. oppositifolius* and *F. vulgare*) did not show antimicrobial activity. Among the spice extracts, extract of *I. verum* was found to be most potent showing highest zone of inhibition against all test organisms, whereas *D. volubilis* did not show antibacterial activity against microorganisms except *B. cereus*. The MID value of *I. verum* extract against *B. cereus, B. pumilus, S. marcescens* and *P. aeruginosa* was found to be 1.25, 2.5, 3.5 and 1.5 mg extract $disc^{-1}$ respectively.

- Anti-quorum sensing activities of different herbs and spices evaluatedthrough preliminary screening for inhibition of violacein synthesis by whole plant parts.Among the plant tested *I. verum* was found to be most potent in inhibiting the violacein production in *C. violaceum,* followed by *P. perlata*. Methanolic extract of *I. verum* was also able in reducing the virulance phenotypes such as pyocyanin synthesis, ptotease production, swarming motility and biofilm formation in *Ps. aeruginosa*.

- Evaluation of *in vivo* anti-diabetic activity was performed in Streptozotocin-induced rats using *I. verum* and *G. oppositifolius* methanolic extracts as they were traditionally reported as antidiabetics.
  - The methanolic extracts were reconstituted in sterile distilled water and used to determine the toxicity and pharmacological effects on rats.
  - Before performing *in vivo* assay, the crude extracts were tested fot their acute toxicity at a concentration of 2000 mg kg^{-1} BW and analyzed the lethal and safer doses of extracts.
  - For anti-diabetic assay 500 mg kg^{-1} BW and 250 mg kg^{-1} BW doses were selected as safer and non-toxic.
  - Streptozotocin-induced diabetic rats treated orally with both the sample extracts and Metformin were able to reverse the diabetic conditions to near normal.
  - Various biological markers such as fasting blood sugar level, cholesterol, triglycerides liver enzymes (SGPT and SGOT), serum urea and creatinine were reduced to nearly normal level while significant increase in body weight and HDL-cholesterol level was observed in compared to the diabetic controls.
  - Among the plant extracts, *I. verum* extract (*Iv*ME) showed comparatively better *in vivo* antidiabetic activity than *G. oppositifolius* extract (*Go*ME). This may due to various antidiabetic compounds presents in the extract.

- Further, characterization of bioactive compounds present in the different fractions of *I. verum* (*Iv*ME) and *G. oppositifolius* (*Go*ME) were performed by GC-MS analysis. GC-MS profiling revealed the presence of multitude chemical

compounds containing volatile compounds, phenolics, terpenoids, fatty acids, phytosterol etc.

- Compounds identified through GC-MS analysis of methanolic fraction of *I. verum* wereLinalool, Estragole, Benzaldehyde, 4-methoxy-; Benzene, 1-methoxy-4-(1-propenyl)- or cis-Anothole; 2-Propanone,1-(4-methoxyphenyl)-; n-Hexadecanoic acid; Benzhydrazide,4-methoxy-N2-(2-trifluoroacetylcyclohepten-1-yl)-; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-, cis-Vaccenic acid; Octadecanoic acid.
- Some of the compounds identified through GC-MS analysis of hexane fraction of *I. verum* were Benzaldehyde, 4-methoxy-; Anethole; Anisaldehyde dimethyl acetal; 2-Propanone, 1-(4-methoxyphenyl)-; 4-(p-Methoxyphenyl)-1-butanol; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene; n-Hexadecanoic acid; cis-Vaccenic acid; (2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-;4-Methoxy-benzoic acid N'-[2-(4-methoxy-phenyl)-acetyl]-hydrazide and Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)-.
- Some of the compounds identified through GC-MS analysis of ethyl acetate fraction of *I. verum* were Linalool; Estragole; Benzene, 1-methoxy-4-(1-propenyl)-; 2-Propanone, 1-(4-methoxyphenyl)-; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene; n-Hexadecanoic acid; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel- and cis-Vaccenic acid.
- GC-MS analysis of different fractions of *G. oppositifolius* also revealed different types of chemical compounds. Compounds identified through GC-MS analysis of methanolic fraction of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; 1-Deutero-2,2,5,5-tetramethylcyclopentanol; n-Hexadecanoic acid; Phytol; 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; (1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydro cyclopropa [d]-naphthalene and Retinol, acetate.

- Compounds identified through GC-MS analysis of hexane fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; Mome inositol; Hexadecanoic acid, methyl ester, n-Hexadecanoic acid; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; Phytol; Linoelaidic acid; Octadecanoic acid; Squalene and Chondrillasterol.
- Compounds identified through GC-MS analysis oethyl acetate fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; DL-Proline, 5-oxo-, methyl ester; Mome inositol; n-Hexadecanoic acid; Phytol; Lanosterol; Lup-20(29)-en-28-ol; Beta.-copaen-4 .alpha.-ol and Retinol, acetate.

❖ Finally, it can be concluded that the two selected spice and herb showed high potential in the different activities tested. *I. verum*, being used as a spice has a large number of volatiles which would be responsible for the flavour and *G. oppositifolius*- a commonly consumed leafy vegetable with so many different phytochemical constituents contributed to beneficial wholesomeness. The health benefits and therapeutic potentials of the investigated herbs and spices might be attributed to the presence of various phytochemical compounds in plant extracts.