Chapter 6

SUMMARY AND CONCLUSION

The review on Alzheimer’s diseases gives the information of understanding the thing concerning risk factors, sign and symptom, diagnosis, current research therapeutic strategies for AD treatment, role of acetylcholine and acetylcholinesterase inhibitors and drugs treatments for Alzheimer’s diseases finding new drug molecules for Alzheimer’s disease and alternative approaches to Alzheimer’s disease Prevention.

A general survey on medicinal plants summarizes information regarding the effective and therapeutic potential ability of herbal medicine. Based on the present research and development, current medical management of AD suggesting that acetylcholinesterase inhibitors slow down or prevent AD. Literature of medicinal plants which are already functioning for AD provides information about the therapeutic use and herbal originated drugs may lead to a discovery of novel medicines.

Based on the literature survey three medicinal plants namely (*Alpinia purpurata*, *Mimusops elengi* and *Rauvolfia tetraphylla*) were selected. After collection, plants were subjected to authentication, cleaning and powdering procedures. Then subjected to different types of extraction techniques to get more yield of crude extracts, Thus soxhlet extraction method only we got good yield compared to other technique like maceration, infusion, and sonication, Hence soxhlet extraction process was adopted and details of yield in grams were reported and stored the crude extracts for further use. Phytochemistry illustrates concerning the huge number of secondary metabolic compounds originate in plants. The crude extracts so obtained were subjected to phytochemical screening tests for the identification of different phytoconstituents like alkaloids, carbohydrates, flavonoids, glycosides, saponins, and tannins by standard methods, to know that which
classes of compounds are present in different solvent extracts. Phytochemical screening of different solvent crude extracts of all three medicinal plants (*A. purpurata, M. elengi* and *R. tetraphylla*) revealed that it was a greater source for the assortment of phytoconstituents. The result of phytochemical screening illustrates the presence of hopeful sources of both primary and secondary metabolites.

The preliminary screening to find out the antibacterial activity of crude extracts of three medicinal plants was carried out against three bacteria viz., *Staphylococcus aureus* (gram-positive), *Escherichia coli* and *Pseudomonas aeruginosa* and three fungi viz., *Penicillin chrysogenum, Cladosporium oxysporum* and *Candida albicans* by using the cup plate method. From the results, we observed that the *R. tetraphylla* methanol extracts and *M. elengi* methanol extracts showed significant antimicrobial activity when compared with the standard Amoxicillin. The antifungal results show that the *A. purpurata* and *R. tetraphylla* methanol extracts showed significant antifungal activity when compared with the standard fluconazole. These above significant crude extracts may be used in traditional medications to treat a variety of infections caused by preferred microorganisms.

The results of HPTLC fingerprint profiling helps to the identified number of phytocompounds in different solvent crude extracts of all three medicinal plants (*A. purpurata, M. elengi, and R. tetraphylla*). In the Hptlc method a better separation was achieved. The visualization reagents enable to see the spots efficiently and the densitometry will able to quantify the constituents. The Rf values, Max Rf and percentage area of densitogram and number of spots were recorded for all crude extracts. If number of spots were identified in densitogram indicated that more number of
bioactive compounds were present in that particular extract, overall, it can be justified that use HPTLC method allows checking major phytoconstituents present in different solvent extracts and their concentration and fingerprint images will be helpful in the identification of unknown bioactive compounds with bio-activity and ensure therapeutic efficacy.

The different solvent crude extracts of all three medicinal plants (A. purpurata, M. elengi, and R. tetraphylla) were subjected to the total antioxidant activity, where antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated by a standard graph of ascorbic acid, where Butylated hydroxyanisole (BHA) was used as reference standard. The methanol extracts of R. tetraphylla and M. elengi showed very good total antioxidant activity. The study showed that all extracts exhibited increased antioxidant activity or decreased pro-oxidant activity with escalating concentration. However, their activities differed according to the type of extract added to the system. The overall, results suggested that solvent all crude extracts were supportive in preventing or slowing development of different oxidative stress-related diseases.

The results of the AChE inhibitory activities of all three plants extracts contained a various level of inhibitory activity against AChE. Different solvents extracts (n-Hexane, DCM, and methanol) were used for this investigation. The IC$_{50}$ values of the plant extracts indicating AChE inhibitory activity with low IC$_{50}$ value is indicative of good inhibition of the enzyme. The IC$_{50}$ for AChE inhibition activity was lowest for R. tetraphylla methanol extract with values 15.26 ± 0.11, R. tetraphylla seed methanol extract 16.49 ± 0.36, A. Purpurata methanol extract 19.08 ± 0.02, M. elengi methanol extract 20.54 ± 0.81 μg/ml. However, the methanol extracts had better activity than the
other solvents extracts, methanol extracts showing the low IC$_{50}$ values percentage inhibition of AChE. The higher activity of methanol extracts may suggest that organic solvent methanol is able to extract more active compounds with possible AChE inhibitory activity than other solvents.

The major intend of isolation of active compounds from medicinal plants are to achieve therapeutically desired compounds to alleviate AD. According to results of the phytochemical screening, antibacterial, antifungal and total antioxidant activity. HPTLC finger print analysis of methanol crude extracts of all selected medicinal plants gives the assessment of a number of phytocompounds. Acetylcholinesterase inhibition activity indicated that the methanol crude extracts of all selected medicinal plants show potential inhibition activity. This observation prompted us to isolate and identify phytocomponents present in this extracts. Present work explains advanced isolation techniques were carried out to eliminate most of the superfluous matrix, which leads to isolate active pure compounds, this chromatographic method development and strategies were empowered by several chromatographic techniques like, Flash chromatography, Pre-HPLC, and HPLC. However, by following above chromatographic techniques to an approach extensively.

Finally, from each medicinal plant two compounds were isolated and structural elucidation was done by spectral studies such as UV, IR, $^1$H NMR, $^{13}$C NMR and mass spectrum. Based on the spectral data and earlier kinds of literature, it was confirmed that A.P. pure compound-1 was zingerone, A.P. pure compound-2 was curcumin, M.E pure compound-1 was lupeol, M.E. pure compound-2 was quercetin, R.T. Pure compound-1 was 2-Methoxy -3-aza-bicyclo [4, 1, 0] hepta-4, 6-diene-4-carboxylic acid, a novel
phytocompound was isolated and characterized and R.T. pure compound-2 was gallic acid. Hence, these phytocompounds have already potent therapeutic properties. But one novel and remaining phytocompounds subjected to acetylcholinesterase inhibition activity and antimicrobial studies for better understanding of their pharmacological application to alleviate AD.

The isolated compounds form all three medicinal plants (A. purpurata, M. elengi, and R. tetraphylla) were screening for Acetylcholinesterase inhibition activity and antimicrobial studies. Natural products have played a significant role as new chemical entities (NCEs) approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived. Another 20% of new chemical entities during this time period were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural product. Natural products provide a starting point for new synthetic compounds, with diverse structures and often with multiple stereo centers that can be challenging synthetically. Many structural features common to natural products (e.g., aromatic rings, complex ring systems, chiral centres, degree of molecule saturation, ratio and number of heteroatoms) have been shown to be highly relevant to drug discovery efforts. Drugs derived from medicinal plants can serve not only as new drugs themselves but also as drug leads suitable for optimization by medicinal and synthetic chemists. When new chemical structures are not found during drug discovery from medicinal plants, known compounds with new biological activity can provide important drug leads. Since the sequencing of the human genome, thousands of new molecular targets have been identified as important in various diseases. With the onset of high-throughput screening assays directed towards these targets, known
compounds from medicinal plants may show promising and possibly selective activity. Several known compounds isolated from traditionally used medicinal plants have already been shown to act on newly validated molecular targets, other known compounds have also been shown to act on novel molecular targets, thus reviving interest in members of these frequently isolated plant classes.

All six isolated phytocompounds exhibited good acetylcholinesterase inhibition activity. Overall results of acetylcholinesterase inhibition activity proved that A.P. pure compound-2 (curcumin) and R.T. pure compound-1 (novel phytocompound) exhibits Low IC$_{50}$ and most effectively inhibition ability followed by quercetin, zingerone, lupeol and gallic acid. Curumin and novel phytocompound may be used as a source of natural acetylcholinesterase inhibitors in order to substitute the synthetic ones.

All isolated phytocompounds illustrates significant antimicrobial activity against all the tested pathogens. Antibacterial activity of the tested phytocompounds was expressed significant activity against the tested bacteria pathogens. Curumin, zingerone, novel phytocompound and quercetin possessed higher antibacterial ability followed by gallic acid and lupeol. Curumin exhibited good and maximum antifungal activity of p. aureginosa (19 mm). Antifungal activity of the tested phytocompounds was expressed significant antifungal activity against the tested fungal pathogens. Highest antifungal activity was observed in Zingerone, Curumin, Novel phytocompound and quercetin followed by gallic acid and lupeol. Zingerone exhibited good and maximum antifungal activity of C. albicans (18 mm). The antimicrobial results of isolated compounds suggested great interest in the development of antimicrobial drug also.
In conclusion, natural products discovered from medicinal plants have provided numerous clinically used medicines. Drug discovery from medicinal plant has traditionally been lengthier and more complicated than other drug discovery methods, hence in our research we isolated the phytocompounds by adopting better methodologies for plant collection, extraction, bioassay screening of acetylcholinesterase activity and innovative chromatographic techniques which leads our speed of active compound isolation by flash chromatography and Prep-HPLC, HPLC and structural elucidation of phytocompound by spectral studies like UV, IR, NMR and Mass spectra to facilitate identification of phytocompounds. The isolated natural products were in small quantities that are insufficient for lead optimization and clinical trials. Our research results revealed that *R. tetraphylla* and *A. purpurata* medicinal plants are the natural rich source of phytocompounds, antioxidants, antimicrobials and acetylcholinesterase inhibitors. Two potent acetylcholinesterase inhibitors were isolated from *R. tetraphylla* and *A. purpurata* finding the ethnomedical use of curcumin and (R.T. pure compound-1) novel phytocompound to promote good health in traditional medicine and development of new drugs to treat the AD. Hence, these natural acetylcholinesterase inhibitors as potential pharmacological agents and a new promising source for management of Alzheimer’s disease.