CHAPTER - 5
SUMMARY AND CONCLUSION

The present study was envisaged to systematically validate two selected medicinal plants designated as bitters in relation to their traditional anti-diabetic claim. *Clerodendrum phlomidis* and *Nymphaea stellata* were selected for the study. Based on the available literature and earlier hypoglycemic reports, leaf was selected as the morphological part of study for both plants. Leaves of *Clerodendrum phlomidis* were collected from out fields of Trichy city, Tamilnadu, India, and the leaves of *Nymphaea stellata* were collected from suburb ponds of Vadodara city, Gujarat, India. Both the plant materials were authenticated by Prof. Daniel Mammen, Botany Department, The M.S. University of Baroda, Vadodara.

Quality control parameters like foreign matter, ash value, extractive value, loss on drying, bitterness value, elemental analysis and detection of microbial contamination were determined as per WHO guidelines to ensure the quality of the raw materials. Morphological, anatomical, histological and powder microscopical studies were carried out and the observations were compared with available literature. The coarsely powdered leaf materials of *C. phlomidis* and *N. stellata* were extracted with solvents of increasing polarity viz., petroleum ether, benzene, diethyl ether, chloroform, ethyl acetate, acetone, methanol and water. All the extracts were studied for the presence and absence of different secondary metabolites by preliminary qualitative phytochemical tests.

5.1 *Clerodendrum phlomidis*

There exists a controversy if agnimantha/arani is *C. phlomidis* or *Premna integrifolia*. To ascertain its identity from the other claimant, DNA sequence for internal transcribed spacer (ITS) region of *C. phlomidis* was determined. The cladistic analysis of the DNA sequence showed that *C. phlomidis* belongs to clade II (African clade), which is contradictory to the previous finding which placed *C. phlomidis* in clade I (Asian clade).

Different *C. phlomidis* extracts showed the presence of steroids, phenolics and alkaloids. Adrenaline, lupeol, l-dopa, β-carotene were identified for the first time in leaves by comparative-TLC. These identified compounds along with earlier reported compound β-sitosterol were quantified by TLC. The preliminary chemical tests showed high quantity of amino acids and also positive reaction for alkaloids, hence the identification of amino acids was carried out. The presence of tyrosine, phenylalanine, alanine, valine, leucine, isoleucine, glutamic acid and threonine were identified by co-TLC. Crude polyamine extract showed the presence of numerous polyamine constituents. When crude alkaloid fraction was separated in TLC, it showed no clear individual spots for alkaloids with dragendorffs reagent.

Based on the evidence in the literature and steroids being the major secondary metabolite of *C. phlomidis*, the unsaponified petroleum ether fraction of methanol extract (UPFMCP) was chosen for phytochemical studies. Three compounds designated as CP I, CP II and CP III were isolated from UPFMCP by column chromatography. CP I, CP II and CP III were characterized as 1-hexadecanol, clerosterol palmity ester and clerosterol respectively. TLC methods were also developed for the isolated compounds.

Unsaponified matter of petroleum ether fraction of methanol extract (UPFMCP), residual methanolic extract after petroleum ether fraction (RMECP), crude polyamine extract (CPECP), CP I, CP II and CPIII were studied for antidiabetic activity in STZ-NAD rat model. UPFMCP and CP III showed better results, of which UPFMCP results were relatively comparable to that of metformin.

CP III showed moderate effect on glucose, insulin, hexokinase, glucose-6-phosphatase and glycogen but no increase in body weight. No effect on stimulation of the residual pancreatic mechanism like stimulating insulin secretion from the remnant β-cells or regenerated β-cells was observed. No weight gain

indicated the non-reversal of gluconeogenesis and glycogenolysis. The observed hypoglycemic effect may be due to insulinomimetic effect or by increasing peripheral utilization of glucose or increased glycogen synthase or increased glycolysis.

The insulin releasing effect of β-sitosterol, antihyperglycemic effect of lupeol, antioxidant property of β-carotene and the hypoglycemic effect of CP III may have contributed to the observed synergistic anti-diabetic activity of UPFMCP. Hypocholesterolemic and hypolipidemic of β-sitosterol, β-carotene and lupeol explains the altered lipid metabolism contributing to weight gain observed in UPFMCP. The signs of β-cell regeneration in the histopatholgical study may be due to pancreatic protective effect of β-carotene or due to unidentified compound/s of UPFMCP. Vitamin E and phytol were identified by GC-MS analysis of UPFMCP. The antidiabetic beneficial effect of vitamin E and the glucose metabolism regulation by phytanic acid (a metabolite of phytol) by peroxisome proliferator-activated receptor agonistic activity may have also contributed to the observed anti-diabetic activity of UPFMCP. From the results, it may also be postulated that at least more than one hypoglycemic principle with diversified mechanism of action may be responsible for the anti-diabetic activity of UPFMCP.

Aqueous extract, 50 % methanolic extract, methanolic extract, UPFMCP, RMECP, CPECP (Crude polyamine extract), CAFCP (Crude alkaloidal fraction), β-sitosterol, lupeol, l-dopa, adrenaline, β-carotene, CP I, CP II and CP III were studied for *in vitro* PTP1B inhibition, brine shrimp lethality assay, anti-platelet aggregation activity and anti-acetylcholinesterase study.

In disagreement to the finding of the STZ-NAD model study aqueous extract and CAFCP exhibited higher PTP1B inhibition. CPECP in STZ-NAD model showed indication that it may involve an insulin-independent-mechanism, but conversely

showed 86.39% PTP1B inhibition. Of the compounds studied lupeol and CP III showed highest inhibition. The most active fraction (UPFMCP) in STZ-NAD study fraction showed comparatively moderate inhibition. The presence of lupeol and CP III may be responsible for the inhibitory activity of UPFMCP.

CP III and UPFMCP showed LC_{50} values of 330 and 1130 μg/ml respectively in brine shrimp lethality assay. CP III, CP II, β-carotene, CP I and β-sitosterol may be responsible for the total lethality effect of UPFMCP.

CAFCP exhibited maximum % inhibition of platelet aggregation. UPFMCP also exhibited effective anti-platelet aggregation activity more than aspirin. Except lupeol none of the other compounds tested showed any significant inhibition.

All the extracts tested showed appreciable acetylcholinesterase inhibition. CPECP and CAFCP showed maximum inhibition. UPFMCP showed 47.66% inhibition. Lupeol and CP III may be responsible for the activity of UPFMCP.

5.2 *Nymphaea stellata*

Different *N. stellata* extracts showed the presence of steroids, saponins, phenolics and flavonoids. Lupeol, β-sitosterol, β-carotene, gallic acid, oleanolic acid and betunilic acid were identified for the first time in leaves by comparative-TLC. These identified compounds were quantified by TLC.

Unsaponified matter of petroleum ether fraction of methanol extract (UPFMNS), chloroform fraction of methanol extract (CFMNS) and the left out residual fraction of methanolic extract (RFMNS) were studied for anti-diabetic study. UPFMNS showed comparable results to that of metformin. Lupeol, β-sitosterol, β-carotene, betulinic acid and oleanolic acid may be responsible for the anti-diabetic activity of UPFMNS. Apart from the hypoglycemic effect of oleanolic acid, it also increases hepatic glycogen and insulin levels. Betulinic acid elevates significantly

the plasma hormone levels of insulin. From the results, it may also be postulated that at least more than one hypoglycemic principle with diversified mechanism of action may be responsible for the anti-diabetic activity of UPFMNS.

UPFMNS was chosen for phytochemical studies and NS I was isolated by column chromatography. NS I was characterized as betulinic acid. Aqueous extract, 50 % methanolic extract, methanolic extract, UPFMNS, CFMNS, RFMNS, β-sitosterol, lupeol, β-carotene, gallic acid, oleanolic acid and betulinic acid were studied for *in vitro* PTP1B inhibition, brine shrimp lethality assay, anti-platelet aggregation activity and anti-acetylcholinesterase study. UPFMNS and betulinic acid showed maximum PTP1B inhibition. Lupeol and oleanolic acid may also have contributed for the activity of UPFMNS. Aqueous extract and 50 % methanolic extract showed LC_{50} values of 2760 and 3690 µg/ml respectively in brine shrimp lethality assay. Oleanolic acid exhibited highest lethality of the compounds. Of different fractions only UPFMNS showed moderate inhibition of platelet aggregation and acetylcholine esterase. Betulinic acid, lupeol and oleanolic acid may be responsible for the activity of UPFMNS.

5.3 Conclusion

- The two selected medicinal plants, *Clerodendrum phlomidis* and *Nympheae stellata* designated as bitters have been systematically validated in relation to their traditional anti-diabetic claim.
- Adrenaline, l-dopa, lupeol and β-carotene were identified for the first time from *C. phlomidis* leaves. Similarly lupeol, β-sitosterol, β-carotene, oleanolic acid, betulinic acid and gallic acid were identified for the first time from *N. stellata* leaves.
- CP I (1-hexadecanol), CP II (clerosterol palmityl ester), CP III (clerosterol) and NS I (betulinic acid) were isolated and characterized from unsaponified petroleum ether fraction of methanol leaf extract of *C. phlomidis* and *N. stellata.*

- Analytical methods were developed for the isolated compounds.
- The extracts/fractions and isolated compounds were evaluated for *invivo* anti-diabetic activity. UPFMCP and UPFMNS showed over all better activity then isolated compounds.
- The unsaponified petroleum ether fraction of methanol extract of leaves of *Clerodendrum phlomidis* and *Nympheae stellata* showed anti-diabetic activity, PTP1B inhibition, anti-platelet aggregation and anti-acetylcholineesterase activity. Comparatively *C. phlomidis* fraction (UPFMCP) showed higher bio-activity than *N. stellata* fraction (UPFMNS).
- Both the fractions (UPFMCP and UPFMNS) being anti-diabetic, anti-platelet and anti-acetylcholineesterase could be more valuable in the management of type 2 diabetes preventing the cardiovascular complications and age-related neuro-degeneration.
- The currently marketed drugs for type 2 diabetes are based on the so-called "one-molecule-one-target" paradigm. However, due to the multifactorial pathogenesis of the disease, drugs that hit more than one biological target may offer a better pharmacological approach. UPFMCP and UPFMNS contain more than one active principle with diversified mechanism of action. Based on results of UPFMCP and UPFMNS, the following mechanisms can be speculated;
    - Synergistic multi-target effect.
    - Pharmacokinetic or physicochemical effects based on improved solubility, resorption rate and enhanced bioavailability of one constituent over another.
    - The respective elimination or neutralization of adverse effects of one constituent by the other contained in the fraction, so that altogether a better effectiveness is achieved.