6. SUMMARY

Diabetes is currently a major public health concern, because its incidence and prevalence are elevated and reaching an epidemic proportions. It is characterized by hyperglycaemia that is associated with long-term complications affecting the eyes, kidneys, heart and nerves. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people.

For centuries, plant and plant products have been used for treating various illnesses. Herbs used as medicinal products have been known since ancient times. Herbal drugs play an important role in healthcare programmes especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants to be potential sources of medicinal substances. The plant drugs are frequently considered to be less toxic when compared to synthetic drug. Hence, they play an important role as alternative medicine due to less side effects and low cost. Therefore it has become extremely important to make an effort towards standardization of the plant material that could be used as medicine.

Our study was planned to investigate the hypoglycaemic activity of *Trigonella foenum graecum* and *Pleurotus ostreatus* and to evaluate their therapeutic potency to prevent or delay the onset of diabetes induced renal complications in streptozotocin induced diabetic animal model.
The study was planned and executed in the following phases

**Phase-I**

- Transverse section of *T.foenum graecum* seed showed outermost testa, wide endosperm and central two cotyledons. Radicle present in the upper region of transverse section was separated from the chamber of cotyledons by parenchymatous tissue. Detailed transverse section shows outer most layer, the epidermis, which was covered with thick cuticle. The epidermis is single layered, palisade like, thick walled, having conical projection at the outer side. A light line, linea lucida extends just below the upper conical like projection and vascular bundles and mucilage material are present.

- The section of *P.ostreatus* showed the presence of the mycelium. The mycelium is formed by much branched hyphae uniting to form a network on the substratum. Hyphae are slender, hyaline and septate. Several hyphal mass together into thick twisted strands called rhizomorphs covered by sheath. Basidiocarp shows a fleshy stalk - stipe and an oyster like head or cap, the pileus. The stalk and the head is formed by interwoven mass of hyphae, the pseudoparenchyma. Gills are found in large numbers varying from 300 to 600. Each gill bears innumerable spores – the basidiospores on both the surfaces. Each basidium bears four basidiospores on short and slender stalk called sterigma. Section through a gill shows three distinct region; namely the trama, sub-hymenium and the hymenium.

- The results obtained in the physico-chemical analysis shows that loss on drying, total ash, acid insoluble ash and water soluble extractive contents of the *P.ostreatus* were higher than the *T.foenum graecum*. 
In *T.foenum graecum* the percentage of alcohol soluble extractive value was higher than that of *P.ostreatus*.

In the present investigation, the qualitative analysis of hydro-alcoholic extract of *T.foenum graecum* showed the strong presence of phenols, quinines and triterpenoids. Flavonoids, alkaloids, coumarins and steroids were moderately present. Tannins, and saponins, were weakly present. Amino acids, glycoside/sugar and carboxylic acids were not present.

Phenol, amino acids, steroids and tannins, sugar were moderately present. Flavonoids were weakly present in *P.ostreatus*. Alkaloids, coumarins, quiniones, triterpenoids, saponins, and carboxylic acids were not present. The presence of these compounds explains the medicinal property of the *T.foenum graecum* and *P.ostreatus* extracts.

The phenol content was observed to be high in *T.foenum graecum* and flavonoids, ascorbic acid (Vit-C) and total antioxidant capacity was also observed to be high in *T.foenum graecum* by the quantitative analysis.

The phenols content was 2.5±0.35 mg/gm of extract in *P.ostreatus* and in *T.foenum graecum* it was 4.876±0.04 mg/gm of extract. Total flavonoids content (TFC) of *T.foenum graecum* was (0.489±0.05/g of extract) have and higher concentration of flavonoids when compared to *P.ostreatus* (0.2±0.01 mg/g of extract). The ascorbic acid (Vit-C) content (AAC) was found to be high in *T.foenum graecum* (5.42±0.20 mg/gm of extract) and low in *P.ostreatus* (0.3±0.01 mg/gm of extract). The results also revealed that total antioxidant capacity of *T.foenum graecum* was 192±4.15 mg/g of extract which was comparatively higher than *P.ostreatus* (142±3.13 mg/g of extract).
Phase-II

- The results of the radical scavenging activities like DPPH, Nitric oxide scavenging assay, Hydroxyl radical scavenging assay, ABTS, Superoxide anion, Hydrogen peroxide, Ferrous chelating activity showed that the hydro-alcoholic extract of *T.foenum graecum* was more potent source of natural antioxidants when compared to *P.ostreatus*.

- The HPTLC finger print of hydro alcoholic extract of *T.foenum graecum* showed 7 peaks at 254 and 366 nm. 9 peaks at 620 nm. The HPTLC finger print of hydro alcoholic extract of *P.ostreatus* has showed11 peaks at 254 nm, 3 peaks at 366 nm, and 9 peaks at 620nm.

- The TLC and HPTLC study of *T.foenum graecum* and *P.ostreatus* revealed that the *T.foenum graecum* extract may have many phytochemicals when compared to *P.ostreatus*.

These results indicates that both the extracts may act as natural antioxidant, and also revealed that the hydro-alcoholic extract of *T.foenum graecum* was more potent than *P.ostreatus*.

Phase-III

- GC-MS analysis showed the presence of 17 compounds in *T. foenum graecum* extract whereas in *P.ostreatus* it showed 13 compounds.

- The secondary metabolites of *T.foenum graecum* and *P.ostreatus* which were obtained from GC-MS analysis were used as the ligands for the *insilico* molecular docking study.
The lead molecules of hydro-alcoholic extracts of *T. foenum graecum* (Seventeen compounds) and *P. ostreatus* (Thirteen compounds) were docked using Angiotensin Converting Enzyme (ACE) as the target protein.

Out of the seventeen ligands of *T. foenum graecum* and thirteen of *P. ostreatus* which were docked with the Angiotensin converting enzyme (ACE) seven of *T. foenum graecum* and eleven of *P. ostreatus* were able to form hydrogen bonds with the active site of target protein (*Angiotensin converting enzyme*).

The interactions of these compounds with *ACE* were similar to that of the commercial drugs that are used as *ACE* inhibitors, which are used to treat diabetic nephropathy.

The results of the *insilico* analysis indicates that the secondary metabolites of *P. ostreatus* was more potent in serving an antagonist to *ACE* in a comparable manner with the standard drugs fluvastatin and quinapril.

**Phase-IV**

The investigation was carried out in animal model and the hypoglycaemic potential of the extracts of *T. foenum graecum* and *P. ostreatus* were analyzed in STZ induced diabetic animals.

The antidiabetic activity of the hydro-alcoholic extract of *T. foenum graecum* and *P. ostreatus* was studied on streptozotocin induced diabetes in male Wistar rats.

The groups which were treated with *T. foenum graecum* 400mg/kg body wt extract and *P. ostreatus* 400mg/kg body wt extract for 12 weeks showed significantly low levels of blood glucose, glycosylated haemoglobin, urea, uric acid, creatinine, total protein, albumin,
cholesterol, triglycerides and electrolytes levels and increased level in insulin. MDA level also showed significant lowering when compared to diabetic group. Antioxidant such as SOD, GSH, and Vit-C and haematological parameters reached near normal levels in treated group. The results were compared with standard drug glybenclamide.

- Histopathological changes in organs like kidney and pancreas showed significant improvement in *T.foenum graecum* and *P.ostreatus* treated groups when compared with diabetic rats.

- The results of phase IV revealed that 400mg/kg body weight of both the extracts were potent hypoglycaemic agent.

**Phase-V**

This phase of experiment was designed to study the renal protective effect of hydro-alcoholic extract of *T.foenum graecum* and *P.ostreatus* at a dose of 400mg/kg body weight.

- The rats were induced for diabetes and were left untreated for 8 weeks, to develop nephropathy.

- After 8 weeks of induction the rats were treated with oral administration of hydro-alcoholic 400mg/kg body wt extracts of *T.foenum graecum* and *P.ostreatus* and standard drug glybenclamide, for a period of 12 weeks.

- After the experimental period the groups which were treated with *T.foenum graecum* and *P.ostreatus* showed near normal levels of blood glucose, insulin glycosylated haemoglobin, urea, uric acid, creatinine, total protein, albumin, cholesterol, and triglycerides and
electrolytes levels and MDA level and antioxidant such as SOD, GSH, and Vit-C, haematological parameters when compared with diabetic group. These results were compared with glybenclamide. The effect of *T. foenum graecum* was more profound than *P. ostreatus.*

- Diabetic nephropathy was analyzed in experimental group of rats by histopathology of renal tissues.
- Histopathological changes in organs like kidney, and pancreas showed significant results in *T. foenum graecum* treated groups when compared with diabetic rats.

The results obtained by *invitro* and *invivo* studies revealed that 400mg/kg body weight of hydro-alcoholic extract of *T. foenum graecum* was more potent source of natural antioxidants and act as hypoglycaemic agent. The extract was also capable of postponing the development of renal complications in STZ induced diabetic rats, thereby supporting the usage of *T. foenum graecum* in treating diabetes and its renal complication in folklore medicine.