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Diabetes mellitus, a dreadful endocrine disorder, is one of the most common global diseases afflicting many from various walks of life in different countries. It is a serious health threat affecting millions of people worldwide. Its major cause includes either complete absence of insulin hormone due to auto-immune disorder/genetic defects/abnormal physiology (Type I) or inadequate biological response towards insulin due to down-regulation of receptors (Type II), leading to elevated blood glucose levels. Elevated blood glucose levels if not controlled may cause deleterious effects on multiple organs like kidney, heart, eyes or nerves. At a later stage, diabetic people show chronic metabolic disorder. The chronic impact of untreated diabetes significantly affects vital organs. The complexity of chronic diabetes or lack of awareness leads to sudden onset of diabetes poses a significant risk of occurrence of ketoacidosis and diabetic coma, if untreated/unnoticed respectively. The multi-organ dysfunction syndrome arises through this metabolic disorder can be mitigated/delayed by utilizing holistic approach of herbal drugs.

Allopathic medicines, or otherwise insulin in Type I diabetes, targeting insulin secretion, decreasing effect of glucagon, sensitization of receptors for enhanced glucose uptake etc and in addition, diet management, increased food fiber intake, resistant starch intake and routine exercise aids in managing diabetes mellitus. With the recent resurgence of interest in natural medicines people are turning towards use of medicinal plants and phytochemicals in health care. India has one of the oldest cultural traditions of use of its medicinal flora since Vedic period. Ayurveda, Unani, Siddha and other traditional systems of medicine are one of the oldest systems and utilize large number of medicinal plants.

Plants have been the major source of medicine since ancient times. Ayurveda and other Indian literature mentioned the used of plants in treatment of various ailments. Even today, in rural areas there is a lavish amount of medicinal plants. Folklore medicinal plants are mostly used in such areas. Western medicine are often limited in efficacy and
carry the risk of adverse effects. In addition they are often too costly, especially for the developing world. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to negligible side effects and low cost. Treating diabetes mellitus with more accessible plant derived compounds is a highly attractive segment.

Herbal drugs have been used since the inception of human beings on this. Various geographical locations worldwide have been using their own folklore medicinal plants for the treatment of diabetes mellitus. Use of plant derived compounds for treatment diabetes mellitus, which are easy accessible, are highly attractive option. These plant are time tested and do not require the usual laborious pharmaceutical synthesis seems.

Many herbal plants exhibit significant clinical & pharmacological activity. Researchers are exploring herbal plants and trying to decode its utility for enhancing health standards of human beings. Plants contains multiple active constituents in complex chemical mixtures developed during its growth under various environmental stresses providing a plethora of chemical families with medicinal utility.

Isolation & identification of active constituents from these plants, preparation of standardized dose & dosage regimen can play a significant role in improving the hypoglycemic action. Effective treatment of diabetes is increasingly dependent on active constituents of medicinal plants capable of controlling hyperglycemia as well as its secondary complications. In the past few years, many new bioactive drugs isolated from plants have demonstrated better antidiabetic activity than oral hypoglycemic agents.

The present thesis entitled; “Pharmacological studies on novel anti-diabetic bioactive constituents of some ethno medicinal plants of Mizoram” deals with screening of few common anti-diabetic ethno medicinal plants of Mizoram and the selection and identification of two potential plant(s) for detailed investigation. It also deals with pharmacognostic, phytochemical, pharmacological, antioxidant, α-glucosidase, α-amylase, glucose uptake, GLUT-4 gene expression and pharmacological evaluations.
Among the many plants ethno medicinal widely used by Mizo traditional folklore and tribal populations for the management and treatment of diabetes mellitus, the medicinal plants *M. roxburghianus* and *P. fraternus* were selected for the investigation to explore the potential to treat diabetes mellitus.

The pharmacognostic and phytochemical evaluation of *M. roxburghianus* and *P. fraternus* plant extracts reveals the standard parameters for the quality and purity of herbal drug and also gives information regarding the authenticity of crude drug. The studies showed presence of steroids, saponins, terpenoids, tannins, flavonoids, glycosides and sugars in *M. roxburghianus* and *P. fraternus* plant extract showed the presence of carbohydrates, glycosides, saponins, alkalooids, tannins, fixed oils and fats. Many of these compounds have been shown to produce potent hypoglycaemic, antihyperglycaemic, and glucose suppressive activities (Table 6.1 to Table 6.5). These effects might be achieved by facilitating insulin release from beta pancreatic cells, inhibiting glucose absorption in gut, stimulating glycogenesis in liver and/or increasing glucose utilization by the body.

The GC-MS analysis of phytochemical constituents of *M. roxburghianus* and *P. fraternus* plant extract were characterized. The gas chromatogram showed that the relative concentration of various compounds getting eluted at different retention times. The GC-MS analysis showed the occurrence of high percentage area of bioactive compounds in both the plant extracts. This study is a step towards understanding the nature of active principles in *M. roxburghianus* and *P. fraternus* (Table 7.1 & Table 7.2 and Figure 7.1 & Figure 7.2). GC-MS analysis of *M. roxburghianus* plant extract showed the presence of twenty nine bioactive compounds that could contribute towards the medicinal properties to the plant. Of these 29 constituents, the major chemical components and their % peak area are di-(2-ethylhexyl)phthalate (44.86 %); 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) Ester (44.86 %); 1-Heptadecanamine (7.59%); 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (7.35%); 1-Hexadecanamine (4.69%); 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- (4.01%); 4,4a,5,6,7,7a,8,9-Octahydro-5a-hydroxy-4a,8a-dim ethylazuleno[6,5-b]furan-3-carboxylic Acid Methyl Ester (2.17%); Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester (2.02%);
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E) (1.97%); Hexanedioic acid, dioctyl ester (1.71%); Benzamide, N,N-diethyl-3-methyl-(1.56%); Pentadecanoic acid, 14-methyl-, methyl ester (1.66%); and Methyl 8,14-Epoxy-15-hydroxy-16-nor-pimarate (1.53 %).

GC-MS analysis of *P. fraternus* plant extract showed the presence of fifty six bioactive compounds that could contribute towards the medicinal properties to the plant. Of these 56 constituents, the major components were 1,2-bis[3',4'-Dimethoxybenzyl]-1,2-bis(methoxymethyl)ethane (66.40%); Phyllanthin (66.40%); 12,13-seco-1,12-epoxy-16-methoxy norditerpenoid Alkaloid (13.91%); 1-[(4'å)-3'-ethylenedioxy-18'-norkaur-15'-en-17'-yl]pyrrolidine (4.00%); 3á-(Peroxymethyl)-5-vinyl-A,B-bisnor-5á-cholestane (4.00%); 2-Allyloxy-1-((1,1-dimethylpropyl)-4-n-pentadecylbenzene (4.00%); 2,3,3',4'-tetramethoxy-5-(3-methoxyprop-1-ethyl)-â-methylstilbene (2.92%); 7-diyethylaminio-3-heptafluoropropyl-4-methylcoumarin (2.92%); 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-(1.54%); Phytol (1.43%); 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(1.43%); 5,10-Dihexyl-5,10-dihydroindolo[3,2-b]indole-2,7-dicarbaldehyde (1.12%); 1,5-Dimethoxy-2,4-bis(3-methylphthalalidyl)benzol (1.12%); Hexadecanoic acid (1.11%); n-Hexadecanoic acid (1.11%); Lucenin 2 (0.49%); Quercetin 7,3',4'-Trimethoxy (0.28%); and psi, psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy- (0.28%).

The phyto compounds of *M. roxburghianus* and *P. fraternus* plant extract were identified with molecular formula and structure, which may be used for drug development.

A HPLC method was successfully developed for fingerprint analysis of *M. roxburghianus* and *P. fraternus* plant extract. The fingerprint obtained provides a good repeatability in separation pattern which demonstrated that the fingerprint presented is a rapid, reliable and effective method suitable for either qualitatively or quantitatively determination of the constituents present in *M. roxburghianus* and *P. fraternus* plant extract. The result of the study demonstrated confirmed the presence of the potential chemical content of Beta-sitosterol and Gallic acid in *M. roxburghianus* plant extract while the peaks of *P. fraternus* plant extract confirmed the presence of Beta-sitosterol, Gallic acid, Quercitin and Rutin (Table 8.1 to Table 8.5 and Figure 8.1 to Figure 8.15).

The *in vitro* antioxidant activities of *M. roxburghianus* and *P. fraternus* plant extracts against DPPH, ABTS, lipid peroxidation assay, and nitric oxide. Both *M. roxburghianus*
and *P. fraternus* plant extract exhibited significant antioxidant property. The *M. roxburghianus* plant extract have shown the significant result in DPPH scavenging assay with IC$_{50}$ 64.58 compared to *P. fraternus* plant extract which showed IC$_{50}$ of 116.67. In nitric oxide scavenging assay, *M. roxburghianus* plant extract showed significant result with IC$_{50}$ 208.33 compared to *P. fraternus* plant extract with an IC$_{50}$ of 394.17. *M. roxburghianus* plant extract were found to possess significant radical scavenging and antioxidant property compared to *P. fraternus* plant extract. These observation shows that antidiabetic activity of the plant extract may also be attributed to the antioxidant property of the plant extract (Table 9.1 to Table 9.9 and Figure 9.1 to Figure 9.8).

The *in vitro* $\alpha$ - glycosidase and *in vitro* $\alpha$ - amylase inhibitory activity of *M. roxburghianus* and *P. fraternus* plant extracts respectively was investigated in this study. $\alpha$ - glucosidase and $\alpha$ - amylase inhibitors regulate postprandial hyperglycemia (PPHG) by impeding the rate of carbohydrate digestion in the small intestine and thereby hampering the diet associated acute glucose excursion. PPHG is a major risk factor for diabetic vascular complications leading to disabilities and mortality in diabetics (Shihabudeen et al., 2011). *P. fraternus* and *M. roxburghianus* has been used in traditional medicine for treating diabetes. In this study we have evaluated the $\alpha$-glucosidase inhibitory potential of *P. fraternus* and *M. roxburghianus* extract. The results of the study indicate that *P. fraternus* plant extract exhibited maximum $\alpha$ - glucosidase and $\alpha$ - amylase inhibitory activity. The plants may essentially contain herbal bioactive compounds inhibiting enzyme activity. One of the mechanism of action of the plant extract as anti-diabetic agent could be by inhibiting the activity of intestinal $\alpha$ - glucosidase and $\alpha$ - amylase (Table 10.1, Table 10.2, Table 11.1 and Table 11.2 and Figure 10.1, Figure 10.2, Figure 11.1, Figure 11.2 and Figure 11.3).

The *in vitro* effect of *M. roxburghianus* and *P. fraternus* plant extracts for their effect on glucose uptake in L6 cell line was investigated. While both the plant extract have *in vitro* cytotoxicity activity and an effective glucose uptake potential, *P. fraternus* plant extract shows better potential compared to *M. roxburghianus* plant extract. From the present findings, it can be concluded that *P. fraternus* plant extract shows moderate toxicity against L6 cell line. The results obtained in the present study clearly demonstrate that
the *P. fraternus* plant extract enhances glucose uptake under *in vitro* conditions. This may be due to its effect on the number of receptors located in the skeletal muscle cell line. The glucose uptake activity of *P. fraternus* plant extract may be attributed to the GLUT4 translocation (Table 12.1 & Table 12.2 and Figure 12.1 & Figure 12.2).

This was confirmed by the *in vitro* study of the effect of *M. roxburghianus* and *P. fraternus* plant extracts on GLUT-4 gene expression in L-6 myotubes cell line. The CTC_{50} value of *M. roxburghianus* plant extract in L6 cell lines indicate that the maximum cytotoxic effect of *M. roxburghianus* plant extract showed 50% reduction in cell viability upon treatment with highest dose (>1000 µg/ml) while *P. fraternus* plant extract showed 50% reduction in cell viability upon treatment with highest dose (593 µg/ml). The results indicate that *P. fraternus* plant extract showed a potent activity against the *in vitro* L-6 cell line compared to *M. roxburghianus* plant extract. *P. fraternus* plant extract demonstrated the up-regulation of GLUT-4 glucose transporter genes, promoting glucose uptake, improving glucose utilization and disposal in skeletal muscles thereby confirming high potential of *P. fraternus* plant extract in the management of Type 2 diabetes mellitus. *P. fraternus* plant extract can be used to reduce insulin resistance associated with Type 2 diabetes mellitus since the plant possess good insulin sensitizing properties (Table 13.1 & Table 13.2 and Figure 13.1 to Figure 13.3).

The *in vivo* acute oral toxicity study of *M. roxburghianus* and *P. fraternus* plant extracts was investigated. This study was conducted to check the non-toxic nature of the plant extracts. The LD_{50} value of *M. roxburghianus* and *P. fraternus* in female rats after single oral treatment is above 2000 mg/kg body weight and is classified as Category 5. The plant extracts were found to be safe can be further explored for their therapeutic potential in different chronic diseases (Table 14.1 to Table 14.6 and Figure 14.1 to Figure 14.4).

*In vivo* anti-diabetic activity of *P. fraternus* plant extract against streptozotocin induced diabetes in rats is was investigated. The results suggests that the *P. fraternus* plant extract has beneficial effect on blood glucose level and ameliorative effect on regeneration of pancreatic islets and may be used as a therapeutic agent in the management of diabetes mellitus. The results proved that the *P. fraternus* plant extract resulted in the control over muscle wasting as a result of the glycemic control. *P. fraternus* plant extract
exhibited significant hypoglycemic effect, significant increase in the activities of SOD, GSH and CAT in STZ induced diabetic rats. significant decrease in LPO (TBARS), significantly lower levels of SGOT, SGPT and ALP (Table 15.1 to Table 15.4 and Figure 15.1 to Figure 15.18).

*P. fraternus* plant extract possess potent antidiabetic property as evident from these studies performed. Further elaborate studies needs to be performed to throw light on the mechanism and confirmation and isolation of active components responsible for the antidiabetic effect of the drug.