CHAPTER – III

ANTIDIABETIC & WOUND HEALING EFFICACY OF
Phragmites vallatoria LEAF ETHANOLIC EXTRACT ON
STZ-INDUCED DIABETIC RATS
Introduction

Phragmites vallatoria a common read belongs to the family of poaceae. It is a huge perennial grass originate in wetlands throughout temperate and tropical regions of the world. Mostly growing in moist fields in tropics of Asia, Africa and Australia. Our interactions with the local tribal community and available literature it has different types of application in medicine and agriculture products.
Young and matured inflorescence of *P. vallatoria*

*Figure 4 P.vallatoria plant photos*

*Phragmites vallatoria* has several medicinal applications such as diuretic, animistic, diaphoretic, wound healing, diabetes, arthritis, rheumatism, antiemetic and febrifuges activities (Naga Vamsi Krishna *et al.*, 2012). It is interesting to note that so far no authentic reports have been found quoting the medicinal properties of *Phragmites vallatoria* and ours is the first scientific study on this plant. Figure 5 explains distribution of the *Phragmites* throughout the world with different colors.

![Distribution map of Phragmites](image)

(Courtesy: Lambertini *et al*. AoB PLANTS 2012; 2012:pls020, Published by Oxford University Press)

*Figure 5: Distribution of the Phragmites throughout the world. P. mauritianus* (pink), *P. australis* (green), *P. frutescens* (purple), *P. japonicus* (yellow), hybrids of *P. mauritianus × P. australis* (red) and hybrids of *P. australis × P. mauritianus* (orange), *P. karka* (blue).
Taxonomy of *Phragmites vallatoria*

- **Domain:** Eukaryota–(Whittaker and Margulis, 1978)
- **Kingdom:** Plantae - (Scamardella, 2010)
- **Subkingdom:** Viridaeplantae
- **Phylum:** Magnoliophyta–(Sinnott, 1935)
- **Subphylum:** Euphllophytina
- **Infraphylum:** Radiatopses–(Kenrick and Crane, 1997)
- **Class:** Liliopsida
- **Subclass:** Commelinidae–(Takhtajan, 1980)
- **Superorder:** Poanae–(Small and Rydberg, 1903)
- **Order:** Poales–(Small and Rydberg, 1903)
- **Family:** Poaceae–(Barnhart and John Hendley, 1895)
- **Genus:** Phragmites– (Oxelman et al., 2000)
- **Specific epithet:** vallatoria - (L.) Veldk.

**Botanical name:** - *Phragmites vallatoria* (L.) Veldkamp

The present study was taken up to confirm the antihyperglycemic activity and wound healing activity of leaf ethanolic extract of *Phragmites vallatoria*.

**Materials and methods & Results**

**Sample collection and estimation of blood glucose**

Blood samples are collected by retro-orbital plexus puncture method and blood glucose levels are estimated using one touch glucometer (Kesari et al., 2006)

**TLC analysis of leaf extracts of *Phragmites vallatoria***

Solvent extraction have been carried out with various compounds we used various solvents such as *n*-Hexane, Ethyl acetate, Ethanol, Chloroform and *n*-Butanol. Thin Layer Chromatography (TLC) is performed with the various solvent extracts to analyze the compound present in the solvent and the spots are observed by exposure of the plates to iodine vapor. Results are presented in Figure 6. Based on the migration of compounds from the point of application (base line) $R_f$ values have been calculated for different solvent extracts and recorded $R_f$ values range from 0.59 to 0.74 (Table 7).
1. TLC of n-Hexane extract of Phragmites vallatoria Leaves
2. TLC of Ethyl acetate extract of Phragmites vallatoria Leaves
3. TLC of Chloroform extract of Phragmites vallatoria Leaves
4. TLC of Ethanol extract of Phragmites vallatoria Leaves
5. TLC of n-Butanol extract of Phragmites vallatoria Leaves

Figure 6: Thin Layer Chromatogram of Different solvent extracts of Phragmites vallatoria

Table 7: TLC analysis of the Different solvent extracts

<table>
<thead>
<tr>
<th>Plant Extract Formulation</th>
<th>$R_f$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.5)</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>0.69</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.72</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.59</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.74</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>0.60</td>
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</table>

Based on $R_f$ value it is speculated that the highest $R_f$ value is due to presence of more number of phytochemical constituents. To further characterize the occurrence of phytochemical compounds present in the leaf extract we have performed a battery of biochemical tests. Hexane extracts confirms the presence of terpenoids, saponins and
resins, negative to rest of the phytoconstituents. Ethyl acetate extract gave positive results for phytosterols, terpenoids, tannins and carbohydrates and rest of the phytoconstituents are negative. The chloroform extract gave positive results for terpenoids and tannins and negative to phytosterols, flavonoids, alkaloids, saponins, glycosides, carbohydrates, proteins, phenols and resins. The n-Butanol extract gave positive results for phytosterols, flavonoids, glycosides and carbohydrates, negative to rest of the phytoconstituents. The ethanolic extract of the plant showed the presence of both polar and non polar phytoconstituents. The ethanol extract confirms the presence of phytosterols, terpinoids, tannins, flavonoids along with the phenols at the same time negative for remaining phytoconstituents. Based on TLC and biochemical analysis it is confirmed that ethanolic extract may consist of most important phytoconstituents in *P.vallatoria* (Table 8).
Table 8: Identification of phyto-chemical constituents of *Phragmites vallatoria*

<table>
<thead>
<tr>
<th>Solvent Extract</th>
<th>Phytosterols</th>
<th>Terpenoids</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
<th>Saponins</th>
<th>Glycosides</th>
<th>Carbohydrates</th>
<th>Proteins</th>
<th>Resins</th>
<th>Phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Ethyl acetate</td>
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<tr>
<td>Chloroform</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>n-Butanol</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>Ethanol</td>
<td>+</td>
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</table>
Gas Chromatography-Mass Spectrometry Analysis of *P. vallatoria* leaf ethanolic extract

Based on TLC reports (or) phytochemical analysis, leaves of powdered form of *P. vallatoria* is extracted with soxhlet extraction with 100% ethanol to identify the presence of natural compounds present in ethanolic extract. Excess of the ethanol is removed by simple evaporation technique. The final fine form of the crystalline powder is used for GC-MS analysis. GC-MS performed with GC Clarus 500 Perkin Elmer equipment. Compounds are alienated in Elite-1 capillary column (100% Di methyl poly siloxane), 30×0.25×1µm. Samples are injected at a temperature of about 250°C with a split ratio of 10: 1 and a flow rate of helium 1 ml/min. Mass detector turbo mass gold-perkin Elmer is used as detector. The constituents are recognized after comparison with those obtainable in the computer library (NIST ver. year 2005) attached to the instrument and reported. Seven compounds with their corresponding peaks are identified during GC-MS analysis shown in Figure 7.
Figure 7: GC-MS chromatogram of *Phragmites vallatoria* ethanolic leaf extract
Two unsaturated methyl esters, two minor fatty acids, diisoctyl ester, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol and phytol were identified. Major comprised compounds are Hexadecanoic acid ethyl ester, 9, 12, 15-Octadecatrienoic acid, methyl ester (z, z, z)-, 9, 12-Octadecadienoic acid (z, z)-, and minor compounds such as Phytol, Octadecanoic acid ethyl ester, 1, 2-Benzenedicarboxylic acid diisoctyl ester and 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol are also identified.

The results are given in (Table 9). It reveals that fatty acid esters, phytol, linoleic acid and plasticizer compounds are present in the extract.
**Antioxidant activity of *P. vallatoria* leaf ethanolic extract**

In arrange to evaluate the antioxidant activity of *P. vallatoria* leaf ethanolic extract, 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) antioxidant assay is carried out using leaf extracts. DPPH is a constant free radical at 37°C (room temperature) and accepts an electron or hydrogen radical to become a stable diamagnetic particle. The antioxidant activity of ethanolic extract is studied. The DPPH scavenging activity is expressed as IC$_{50}$ values. Results depicted in (Table-10) interpreted that the ethanolic extract of *P. vallatoria* show lowest IC$_{50}$ value with efficient free radical scavenging activity. A potential well known antioxidant, Vitamin-C is used as a reference.

**Table 10: DPPH free radical scavenging activity of *Phragmites vallatoria* leaf ethanolic extract**

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phragmites vallatoria</em> leaf ethanolic extract (EPVL)</td>
<td>735±0.85</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>23±0.66</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M

**Anti-diabetic efficacy of ethanolic extract of *P. vallatoria* on STZ-induced diabetic rats**

In order to study the anti-diabetic (anti-hyperglycemic) activity of ethanolic extract of *P. vallatoria*, wister male rates have been taken as in vivo experimental models. Total of 100 rats are chosen for our experimental studies. For induction of diabetes, rats are left without providing food and water (kept for fasting) for 12 hours, then are intraperitonially administrated with streptozotocin (STZ) at a dose of 55 mg/kg body weight. In order to avoid rapid raise/drop of glucose levels in STZ induced rats have maintained the levels of glucose by providing 5% of glucose in drinking water bottle. Following treatment, blood samples are collected at various time intervals (0hrs, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours and 6 hours) and the levels of Fasting Blood Glucose (FBG) levels are determined using one touch glucometer after the 3 days of the incubation period. Experimental data showed that a total of 42 rats are showed hyperglycemic activity, these animals are used for further analysis to test the antidiabetic activity of *P. vallatoria* leaf ethanolic extract (EPVL). In order to examine antidiabetic effects of *P. vallatoria*, experimental animals are separated into 7 groups keeping at least 6 animals in group as follows.
1) Control Diabetic rats without EPVL
2) Diabetic rats with 100 mg/Kg body weight of EPVL
3) Diabetic rats with 200 mg/Kg body weight of EPVL
4) Diabetic rats with 300 mg/Kg body weight of EPVL
5) Diabetic rats with 400 mg/Kg body weight of EPVL and
6) Diabetic rats with 500 mg/Kg body weight of EPVL
7) Normal control rats without EPVL

Graph 1: Anti hyperglycemic activity of EPVL extract on STZ-induced diabetic rats

Results showed (Graph 1) that diabetic rats (group 1) showed higher fasting blood glucose levels as compared with treated and normal controls. Among several fractions it is noticed that Group 6 (500 mg/Kg body weight of leaf extract) animals dropped the blood glucose levels and it follows regular blood glucose levels of healthy animals, which is evident from the control treated rates.

*P. vallatoria* leaf at a dose of 500mg/kg body weight/day is administered for 63 days. When blood samples are analyzed in all the groups, the levels of fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1C) are decreased in normal rats treated with EPVL when compared to controls. Furthermore Similarly, FBG and HbA1C levels are significantly decreased in EPVL treated diabetic rats than diabetic controls. Therefore, the optimal dose to examine the antidiabetic activity have been fixed at
500mg/kg body weight keeping this dose as minimum dose for antidiabetic activity for further experiments to be carried out.

30 male albino rats are divided into 5 groups with each group containing 6 rats groups are, treated with different formulations as follows:

Group 1 represented the control
Group 2 represented the normal rats treated with EPVL extract of 500mg/kg body weight/day
Group 3 represented the STZ-induced diabetic control rats
Group 4 represented STZ-induced diabetic rats treated with EPVL extract (500mg/kg body weight/day)
Group 5 represented STZ-induced diabetic rats treated by glibenclamide (0.2g/kg body weight/day for 9 weeks)

| Table 11: Study on effect of EPVL on different biochemical parameters in rats |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Body Weight (gm)| 167.2 ± 2.10     | 168.5 ± 1.11     | 150.4 ± 1.28     | 164.1 ± 1.14     | 173 ± 0.99       |
| HbA1c (%)       | 2.87 ± 0.10      | 2.66 ± 0.14      | 3.89 ± 0.20      | 2.71 ± 0.18      | 2.44 ± 0.08      |
| FBG (mg/dl)     | 88.6 ± 2.15      | 83.6 ± 0.58      | 347.5 ± 20.26    | 115.5 ± 1.33     | 78 ± 1.02        |
| Skeletal muscle Glycogen (mg/g) | 8.43±1.90 | 8.38±0.82 | 1.77±0.83 | 6.33±0.74 | 7.13±0.32 |
| Liver glycogen (mg/g) | 43.8±1.29 | 42.9±1.96 | 7.65±0.94 | 25.9±2.33 | 28.1±1.11 |
| Insulin (nano gram/ml) | 1.3 ±0.10 | 1.4±0.03 | 0.5 ±0.04 | 1.1 ±0.05 | 1.2±0.07 |

Data was expressed as a mean ± SEM

With regard to body weight, liver and muscle glycogen levels, are not significant affected difference between group-1 and group-2 rats. However, there is a significant increase in body weight and glycogen levels of diabetic rats treated with *P.vallatoria* leaf ethanolic extract group-4 than diabetic controls group-3. An increase of 3-4 folds in muscle and liver glycogen levels is observed in group-4 rats compared to diabetic controls. Insulin levels in Diabetic control group are decreased when compared with other groups. Group-4 rats showed significant increase of insulin (Table 11). Figure 8 conforms the cataract of diabetic rats compare to the normal rat eyes.
Figure 8: Normal Rat eyes and Diabetic rat eyes

Evaluation of Wound healing activity of *P. vallatoria* leaf ethanol extract in STZ-Induced diabetic rats.

After measuring the anti-diabetic activity of EPVL extract, we have examined, whether, the same extract can heal the wounds in diabetic patients. An experiment is designed and created the dead space and excision models to investigate the wound healing activity of EPVL.

**Materials and methods**

**Experimental Animals**

Mainly animals were distributed into five groups of 6 each in excision and 4 groups of 6 each in dead space wound models.

**Animal groups**

The animals are weight matched (n=6 animals group−1) and placed into 5 groups. Animals in group A and B are normal control and normal treated with Vaseline respectively, in group C & D are the diabetic control and diabetic experimental animals treated with ethanol extract of *P. vallatoria* leaf (EPVL), and group E were positive control treated with Bacitracin ointment. Control animals were injected with
normal saline. Fasting blood glucose levels were measured three days later to confirm the diabetic status of the animals.

For excision wound model animals are anaesthetized with diethyl ether through open mask method and bald on both sides of the dorsal with an electric clipper (Singh and Sharma, 2009). The area of wound to be created is outlined on the dorsal side of the animals with methylene blue using a stainless steel stencil. Animals are closely observed for any infection and those which showed signs of infection are separated / excluded from the study and replaced. Following confirmation, animals are divided into 5 groups of 6 in each. The normal controls group A is applied with Vaseline two times a day. Normal treated group B are applied EPVL extract two times a day. Diabetic controls group C are applied with Vaseline two times a day, diabetic experimental rats group D are applied with EPVL extract two times a day and the positive control group E an application of bacitracin ointment 2 times a day. The treatment is done topically in all cases. Wound areas are measured on day 1, 5th and 10th for all the groups using a transparency sheet and a marker. For dead space wound models wounds are inflicted by implanting sterile cotton pellets, one on either side in the groin and axilla on the ventral surface of each rat. The animals divided into 4 groups of 6 each the normal controls group A provided water orally. Experimental controls group B are given the extract orally in a dose of 400mg kg\(^{-1}\) for 11days, diabetic controls group C are given water orally and diabetic experimental rats group D are given extract orally at a dose of 400mg kg\(^{-1}\) for 11days. On the 11th post wounding day, the granulation tissue formed on the implanted cotton pellets is removed carefully under anesthesia. Noting the wet weight of the granulation tissue the tissue is dried at 60\(^{0}\)C, for 12 hours and the weight is recorded. Results showed that (Table 12) \textit{P. vallatoria} leaf ethanol extract significantly increased in the wound healing activity. The percentage of wound contraction is greater in diabetic animals treated with extract (group D) than in control group C animals (Figure 9).
The dead space wound model was used to study the difference in matrix synthesis between drug treated and control groups. Oral administration of the leaf extract (EPVL) appears to increase the mass of granuloma in both normal as well as diabetic animals.

**Figure 9: Wound healing activity of P. vallatoria leaf ethanol extract in STZ induced diabetic rats (excision wound model)**
Table 12: Effect of ethanol leaf extract of *P. vallatoria* on excision wound model in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Period of study (Days)</th>
<th>Wound area (mm²)*</th>
<th>Group-A (NC)</th>
<th>Group-B (NT)</th>
<th>Group-C (DC)</th>
<th>Group-D (DT)</th>
<th>Group-E (PCTB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day – 1</td>
<td></td>
<td>215.8 ± 1.870</td>
<td>215.6 ± 1.650</td>
<td>216.2 ± 1.328</td>
<td>220 ± 1.686</td>
<td>215 ± 1.64</td>
</tr>
<tr>
<td>Day – 5</td>
<td></td>
<td>98.46 ± 1.23</td>
<td>130.8 ± 1.714</td>
<td>78.60 ± 1.009</td>
<td>105.4 ± 1.538</td>
<td>180.6 ± 1.65</td>
</tr>
<tr>
<td>Day – 11</td>
<td></td>
<td>48.6 ± 1.346</td>
<td>95.12 ± 2.15</td>
<td>44.6 ± 1.203</td>
<td>82.6 ± 1.238</td>
<td>139.6 ± 1.74</td>
</tr>
</tbody>
</table>

*The values are shown as mean ± SE (n=6 animals group⁻¹).

NC: Normal control, NT: Normal treated, DC: Diabetic control, DT: Diabetic Treated, PCTB: Positive Control Treated with Bacitracin.

In the dead space model the extract treated animals in groups B&D showed significant increase in the dry and wet weight of the granulation tissue than the animals treated without the extract was observed (Table 13).

Table 13: Wound healing activity of ethanol leaf extract of *Phragmites vallatoria* in Dead space wound model on STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group-A (NC)</th>
<th>Group-B (NT)</th>
<th>Group-C (DC)</th>
<th>Group-D (DT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Granulation Weight (mg 100g⁻¹ rat)</td>
<td>101.23 ± 5.162</td>
<td>120.4 ± 6.482</td>
<td>86.74 ± 4.816</td>
<td>123.7 ± 7.106</td>
</tr>
<tr>
<td>Dry Granulation Weight (mg 100g⁻¹ rat)</td>
<td>42.36 ± 2.162</td>
<td>39.84 ± 4.219</td>
<td>37.68 ± 1.84</td>
<td>62.49 ± 6.486</td>
</tr>
</tbody>
</table>

* The values are shown as mean ± SE (n=6 animals group⁻¹).

NC: Normal control, NT: Normal treated, DC: Diabetic control, DT: Diabetic Treated

**Discussion**

It is inevitable to qualitatively analyze to know medicinal and pharmaceutical importance of a medicinal plant especially during drug discovery as it gives in sequence about the presence of any particular primary or secondary metabolite in the extracts of the plant which are having a clinical consequence (Vuorela *et al.*, 2004; Joshi, 2012). Different phytochemical experiments conducted on the various extracts of *P. vallatoria* leaves shows (Table 8) the occurrence of phenols, terpinoids,
saponins, alkaloids, flavonoids and phytosterols. The chloroform extract gave positive results for terpenoids and tannins and negative to phytosterols, flavonoids, alkaloids, saponins, glycosides, carbohydrates, proteins, phenols and resins and earlier reports revealed chloroform extract contains terpenoids and tannins (Vijayalakshmi and Ravindhran, 2012). The ethanol extract of the *P. vallatoria* showed the incidence of polar and non polar phytoconstituents especially phytosterols, terpinoids, tannins, flavonoids along with the phenols based on TLC reports and biochemical analysis it is confirmed that the ethanolic extract may consist of most important phytoconstituents. Experimental data on thin layer chromatogram gave excellent results in mobile phase is Toluene: Ethyl acetate: Formic acid ratio but not with Toluene: Ethyl acetate: Chloroform ratio. Hexadecanoic acid, ethyl ester is main in relation with other fatty acids. In the investigated extract α-linolenic and linoleic acid, which belong to the group of so called Essential Fatty Acids (EFAs). In the meantime, the polyunsaturated fatty acid (Z, Z)-9, 12-octadecadienoic acid (LA), a conjugated linoleic acid identified as an antioxidant that can defend membranes from harm, increases β-oxidation in the liver and reduces the levels of total body lipid and liver triacylglycerol and unsaturated fatty acid biosynthetic capability (Salminen and Mutanen, 1998; Zhang et al., 2007). The phytol conversion of phytanic acid is a natural rexinoid (McCarty, 2001; Elmazar et al., 2013). It shows antidiabetic activity in type-II diabetic patient’s Linoleic acid, and their conjugates also can regularize impaired glucose tolerance in zucker diabetic fatty acids (McCarty, 2005). All chlorophyll derivatives are having antioxidant properties (Larson, 1988; Ferruzzi and Blakeslee, 2007).

GC-MS analysis of ethanolic extract of *P. vallatoria* leaf ethanolic extract reveals the presence of fatty acids and plastisizer compounds. Mainly *P. vallatoria* leaf ethanolic extract having the most efficient free radical scavenger by the lowest IC$_{50}$ value. Vitamin-C showed higher activity than that of *Phragmites vallatoria*. The higher DPPH radical-scavenging activity of *Phragmites vallatoria* might be due to the incidence of other chemical constituents rather than fatty acids.

Previous studies (Singh et al., 2007; Cazzola et al., 2011) have proved antidiabetic activity of certain herbal remedies. In diabetes, glycogen content of liver and skeletal muscles usually decrease due to improper regulation of lipid metabolism in diabetic rats (Tan et al., 2005). On the other hand the HbA$_{1c}$ levels are decreased upon treatment with ethanolic extract compared with diabetic treated & untreated rats. HbA$_{1c}$ is produced by glycosylation of hemoglobin and stimulates increased insulin
secretion in STZ-induced diabetic rats (Kumar et al., 2006; Eliza et al., 2009; Vamsi krishna et al., 2012). In the case of body weights a significant increase is observed in normal and diabetic treated rats due to the supplementation of P.vallatoria leaf ethanolic extract processing polyphenolic content (Maiti et al., 2004; Naga vamsi krishna et al., 2012).

HbA$_{1C}$ levels are decreased in both normal rats treated with EPLV and diabetic rats treated with EPLV when compared with diabetic and diabetic untreated rats. This may be mainly due to the decreased FBG levels. Insulin levels are decreased in diabetic control group due to the high FBG level and little gain in EPVLD treated rats due to the decreased FBG & HbA$_{1C}$ levels (Pushparaj et al., 2007). On other hand healing process begins immediately following injuring when the platelets coming to contact with exposed collagen (Monaco and Lawrence, 2003). As platelet aggregation proceeds, clotting factors are released resulting in the deposition of a fibrin clot which serves as a provisional matrix and sets the stage for the subsequent events of healing (Diegelmann and Evans, 2004). P.vallatoria having wound healing activity is proposed by earlier workers by peripheral application of crude extracts.

The dry granuloma weight is decreased by the leaf extract treatment in non diabetic animals and dry granuloma mass is increased by the extract treatment in diabetic animals. Finally the conclusion is significant increase in the wound healing activity is observed in leaf extract treated rats (Naga vamsi krishna et al., 2012; Ghosh et al., 2012). In excision wound model, animals of group B and D showed decrease in the epithelization period and increased percentage of wound contraction when compared with the animals of group A, C, E. In the dead space model the extract treated animals in groups B&D showed significant increase in the dry and wet weight of the granulation tissue than the animals treated without the extract is observed (Geethalakshmi et al., 2013). The present study demonstrated that EPVL extract applied topically promotes healing of wound contraction in STZ induced diabetic rats where healing is delayed.

**Conclusion**

In order to interpret the principle compounds(s) present in the P. vallatoria and to isolate, purify and to elucidate the structure of principle active compound(s) entire plant is screened. Studies clearly indicated that the rhizomes of this plant found to contain maximum concentration of active principle(s). Moreover, it also found that more amount of aliphatic moiety present in the active fraction of P. vallatoria.