Chapter V
Antidiabetic activity of Cymbopogon citratus (DC.) Stapf.
1. Introduction

Several plant components that are grown in the backyard and kitchen garden are known to have very good antidiabetic action. Regular vegetables such as *Mamordica charantia, Moringa oleifera* and fruits such as *Eugenia jambolana, Psidium guajava* etc. are reported to possess good antihyperglycemic action. A large number of diabetic patients all over the world resort to such alternative therapies on world of mouth while some of the plants genuinely possess good biological action while many others just provides a placebo effect. It therefore is very vital to analyze such plants that are reported or used traditionally for their antidiabetic action. It is also important to be able to isolate and identify the active constituents and assign proper mechanism by which they lower the blood glucose. One such plant regularly grown in kitchen garden and used in treatment of several diseases is lemon grass. In this chapter we have attempt to study the antidiabetic activity of the plant and to identify the active constituents present possessing antihyperglycemic action as well as the possible mechanism by which they control the blood glucose.

1.1 *Cymbopogon citratus* (DC.) Stapf.

*Cymbopogon citratus* (DC) Stapf belongs to the family Poaceae and commonly known as ‘lemon grass’. *Cymbopogon citratus* is one of the important sources of essential oils for the flavour and fragrance industries worldwide. It is also greatly used in various traditional medicines as infusion or decoct. The Cuban population has employed the species as an antihypertensive and anti-inflammatory drug (Carbajal et al., 1989). In eastern Nigeria, this plant has been utilized for treating diabetes, obesity and coronary disease (Adeneye and Agbaje, 2007). Tea obtained from leaves of *Cymbopogon citratus* (DC) Stapf is used for its anxiolytic, hypnotic and anticonvulsant properties in Brazilian folk medicine and similar results were observed in laboratory animals (Carlini et al. 1986; Blanco et al. 2009). However, there is report that tea obtained from leaves of *Cymbopogon citratus* does not show hypnotic or anxiolytic effect in humans and also not shows any toxicity (Leite et al. 1986).

The essential oil and its isolated components shown to possess antibacterial activity in various studies. This oil has also been reported to be antimicrobial (Chalchat
The oil shows fungicidal and anti-aflatoxigenic effects against *Aspergillus flavus* Link. (*Paranagama et al.* 2003). The major components of oil as citral-a and citral-b have been shown to possess antibacterial activity (*Onawunmi et al.* 1984). In another study, the antibacterial properties of three main components of the essential oil have been recorded. The α-citral (geranial) and β-citral (neral) components individually elicit antibacterial action, the third component, myrcene, did not show observable antibacterial activity on its own. However, myrcene provided enhanced activities when mixed with either of the other two main components identified (*Grace et al.* 1984).

The antioxidant activity of *Cymbopogon citratus* was assessed in various studies and found to possess good antioxidant activity (*Cheel et al.* 2005; *Melo et al.* 2001; *Koh et al.* 2011). *C. citratus* also shows cytoprotective and anti-inflammatory property by reducing the oxidative stress (*Tiwari et al.* 2010). The lemongrass essential oil protects DNA against chemically-induced damage and also exhibits anticarcinogenic activity against chemically induced mammary carcinogenesis in female Balb/C mice (*Bidinotto et al.* 2010). The oral single dose or prolonged treatment for 21 days of lemongrass (*Cymbopogon citratus*) essential oil does not show any genotoxic or toxic effects in mice and shown to beneficial in reducing the blood cholesterol level (*Costa et al.* 2011).
2. Material and methods

2.1 Plant material

The grass was collected from the Shivaji University, Kolhapur, MS-India and identified by the Department of Botany, Shivaji University, Kolhapur, MS-India.

2.2 Extraction of fractions from steam distillate

The fresh leaves of *Cymbopogon citratus* were cut into small pieces and subjected to steam distillation. The distillate collected was subjected to extraction with petroleum ether (pet ether) for isolation of essential oil (EO). The % yield of essential oil (EO) obtained through pet ether extraction is 0.39% w/w. The essential oil (EO) was stored in airtight screw cap glass vials, protected from light at 4°C until further use. For *in vivo* and *in vitro* experiments, the essential oil (EO) was diluted with dimethylsulfoxide (DMSO) just before the use. The final concentration of DMSO not exceeds than 0.1% in dilution. Vehicle control is used in each *in vivo* and *in vitro* experiment.

2.3 Induction of Diabetes

Diabetes was induced in normal male Wistar rats using streptozotocin as per method mentioned in previous chapter.

2.4 Oral glucose tolerance test (OGTT)

Total 30 rats (6 normal and 24 diabetic) were fasted overnight with free access to water. Initial blood glucose of each rat was measured. They were divided into 5 groups (in each group n=6) as normal control, diabetic control, positive control (glibenclamide 2.5 mg/kg body weight) and remaining groups were as *C. citratus* essential oil (EO) with 10 mg and other with 20 mg/kg body weight. All rats were fed orally with glucose load of 3 mg/gm body weight. The normal and control group rats were administrated orally with vehicle while others with respective test component ten
min prior to glucose administration. Blood glucose was measured with ACCU-CHECK at 0, 30, 60 and 120 min.

2.5 Experimental design

All the experiments were carried out as per the guidelines of the Institutional Animal Ethical Committee after due submission and approval of the protocols. The biological active pet ether fraction was carried out for further prolonged and in vitro studies.

2.6 In vitro studies

2.6.1 Isolation of rat pancreatic islets

The isolation of islets was carried out using collagenase digestion method (Shewade et al., 1999) with some modifications (Patil et al., 2011). The detailed procedure was mentioned in previous chapter.

2.6.2 Insulin release assay

Groups of 10 islets were placed in wells each containing 1 ml HBSS (pH 7.4) supplemented with 10 mmol/l HEPES and 2 mg/ml BSA. Cells were then incubated for 1 h with 11.8 mM glucose in presence of test components. Glibenclamide (20 µM) which is a commercially used sulfonylurea, was used as positive control. Isolated islets were incubated in presence or absence of the test compound in presence of 11.8 mM glucose. After 1 hour incubation of islets in above mentioned conditions, supernatant from each well was collected and stored at -20 °C until further use. The insulin concentration in all the stored samples was determined by ELISA kit (CalBiotech) and quantified by using ELISA microplate reader (Multiskan EX, Thermo Scientific) at 450 nm.
2.6.3 Viability assessment by MTT conversion

The viability of isolated islets after experimental treatment was assessed by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay as detailed in previous chapter. The viability of cells was expressed in percent viability (% viability) relative to vehicle control group islets which were considered as 100% viable.

2.7 Isolation and identification of bioactive compound form pet ether fraction

2.7.1 Isolation of compounds by silica gel column chromatography

The essential oil (EO) of C. citratus was subjected to silica gel column chromatography. The essential oil (EO) was loaded on a silica gel column (60-120 mesh size, 1.2 internal diameter x 50 cm) and successively eluted with stepwise gradient of Hexane: Ethyl acetate system (100:0, 90:10, 80:20, 70:30, 60:40, 40:60, 20:80 and 0:100). Total 8 fractions were collected for this step.

3 Statistical analysis

All the data obtained was expressed as mean ± SD. Statistical analysis was performed using ANOVA and Unpaired Student t-test. A value with $p < 0.05$ was considered as statistically significant while $p < 0.005$ as extremely significant.

4 Results and discussion

4.1 Oral glucose tolerance test for pet ether fraction

The antihyperglycemic activity of C. citratus essential oil (EO) was assessed through oral glucose tolerance test (OGTT) and shown in Table 1. The diabetic rats receiving vehicle are considered as negative control while that with glibenclamide (2.5 mg/kg body weight) are considered as a positive control. In diabetic control group, blood glucose levels rapidly increases from the time of glucose administration and remains abnormally very high even after a period of 120 min as compared to normal control. In rats administrated with glibenclamide (2.5 mg/kg rat body weight), effective control on blood glucose levels was observed. Glibenclamide is a commercially used sulfonylurea
compound used in treatment of diabetes mellitus. The essential oil of *C. citratus* showed antihyperglycemic action in dose dependent manner as compared to diabetic control. At any dose i.e. 10 mg or 20 mg/kg body weight, the antihyperglycemic activity of *C. citratus* essential oil was not as potent as the glibenclamide but effective as compared to diabetic control. The antihyperglycemic activity was due to pet ether fraction of *C. citratus* distillate. Thus, the bioactive pet ether fraction was used for further studies.

### Table 1- Effect of *C. citratus* oil on blood glucose levels

<table>
<thead>
<tr>
<th>Group (Treatment and dose/kg body wt)</th>
<th>Blood glucose (mg/dl)</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>70.3±3.6</td>
<td>106.7±5.0</td>
<td>93.0±4.6</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>253.5±7.6</td>
<td>557.0±5.7</td>
<td>464.2±7.6</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (2.5 mg/kg)</td>
<td></td>
<td>251.2±6.7</td>
<td>370.2±6.2</td>
<td>284.7±6.3</td>
</tr>
<tr>
<td>Diabetic + <em>C. citratus</em> oil (10 mg/kg)</td>
<td></td>
<td>238.5±7.8</td>
<td>457.2±7.0</td>
<td>382.2±8.2</td>
</tr>
<tr>
<td>Diabetic + <em>C. citratus</em> oil (20 mg/kg)</td>
<td></td>
<td>244.7±8.3</td>
<td>426.2±8.3</td>
<td>353.5±7.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=6. All the values are significant with respect to diabetic control (*p*<0.0005).

Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of *Cymbopogon citratus* was previously reported (Adeneye and Agbaje 2007). The authors reported that single, daily oral dosing of 125-500 mg/kg of fresh leaf aqueous extract of *Cymbopogon citratus* for 21 days in normal male rats showed lowering of fasting blood glucose levels and improved lipid parameters dose dependently. In lipid profile, the plasma HDL cholesterol levels were reported to increase while with no effect on plasma triglycerides levels. The results of acute oral toxicity (at a dose of 5000 mg/kg body weight/oral route) in the same study showed that *Cymbopogon citratus* could be considered relatively safe on acute exposure and thus assured its folk medicinal use and safety in suspected type 2 diabetic patients. Though the hypoglycemic action of *Cymbopogon citratus* was mentioned the mechanism for blood glucose lowering effect
and chemical characterization of active constituents was still unknown. Also, the antihyperglycemic potential of essential oil of *C. citratus* was not reported previously. Hence, the present data demonstrates that *C. citratus* essential oil possess good antihyperglycemic activity.

The *in vitro* effect of *C. citratus* essential oil on insulin secretion was assessed to know the probable mechanism action for antihyperglycemic activity of *C. citratus* essential oil.

### 4.2 *In vitro* insulin secretagogue action of *C. citratus* essential oil

The effect of essential oil of *C. citratus* extracted from steam distillate is shown in Figure 1. The isolated islets were challenged with three concentrations of *C. citratus* essential oil viz. 2.5 µg, 5 µg and 10 µg/ml of the reaction mixture in presence of 11.8 mM glucose. Insulin secretion in presence of 11.8 mM glucose was considered as negative control while 10 µg/ml of glibenclamide (a commercially used sulfonylurea) in presence of 11.8 mM glucose was used as positive control. Insulin secretion in each test was expressed in terms of µIU/10 islets/60 min.

![Figure 1. Effect of *C. citratus* essential oil on *in vitro* insulin stimulation](image)

Results are mean ± S.D.; n=6. *p<0.05 and **p<0.005 significant from 11.8 mM glucose control.
Glibenclamide is a commercially used sulfonylurea drug which directly binds and inhibits the $K^+$-ATP channel, thereby depolarizes the beta cell membrane and stimulating $Ca^{2+}$ influx which ultimately leads to insulin secretion. In the in vitro studies on isolated islets, *C. citratus* essential oil showed dose dependent insulin secretagogue activity. The insulin secretion due to essential oil at a concentration 10 $\mu$g/ml was almost 2.06 times higher than that of 11.8 mM glucose control. Though the essential oil showed the dose dependent insulin secretagogue activity, the insulin secretion even at a 10 $\mu$g/ml concentration is less than glibenclamide (10 $\mu$g/ml).

5. Isolation and characterization of bioactive component/s from pet ether fraction

5.1 Isolation of compounds by silica gel column chromatography

The petroleum ether fraction was separated on silica gel column chromatography using Hexane: Ethyl acetate gradient system as 100:0, 90:10, 80:20, 70:30, 60:40, 40:60, 20:80 and 0:100. The % yield (w/w of initially loaded sample) for each fraction was tabulated in Table 2.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Gradient (Hexane: Ethyl Acetate)</th>
<th>% wt of fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100:0</td>
<td>3.74</td>
</tr>
<tr>
<td>2</td>
<td>90:10</td>
<td>36.54</td>
</tr>
<tr>
<td>3</td>
<td>80:20</td>
<td>11.05</td>
</tr>
<tr>
<td>4</td>
<td>70:30</td>
<td>5.88</td>
</tr>
<tr>
<td>5</td>
<td>60:40</td>
<td>3.56</td>
</tr>
<tr>
<td>6</td>
<td>40:60</td>
<td>1.60</td>
</tr>
<tr>
<td>7</td>
<td>20:80</td>
<td>3.55</td>
</tr>
<tr>
<td>8</td>
<td>0:100</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Among these eight fractions, the third fraction i.e. 80:20 and 70:30 were found to be most bioactive in stimulation of insulin secretion. This fraction was further evaluated by using thin layer chromatography using commercially used geraniol and myrcene as a standard reference compounds. The $R_f$ values of two major components observed in TLC of active fraction matches with the $R_f$ values of geraniol and myrcene. Hence, the antihyperglycemic activity and insulin secretagogue activity of both geraniol and myrcene is evaluated.

Both the geraniol and myrcene belongs to class of terpenes. Terpenes with more than 23,000 known compounds are the largest group of natural substances (Wang et al., 2005). Biosynthetically they are derived from isoprene units with the molecular formula (C5H8)n. They are abundantly found in fruits, vegetables, and aromatic and medicinal plants where their important function is protection against infections, parasites and other stress conditions (Bakkali et al., 2008). A variety of monoterpenes and their derivatives have been shown to posses cancer chemopreventive and chemotherapeutic properties (Crowell, 1999). Geraniol and myrcene are acyclic monoterpenes and present in essential oils of many medicinal and aromatic plants that are endowed with many biological activities, including antioxidant, antimicrobial, anti-inflammatory, and antitumor properties (Moteki et al., 2002; Pattnaik et al., 1997; Santos et al., 2004; Tepe et al., 2004). Geraniol and myrcene are mainly used in the manufacturing of cosmetic s, shampoos, toilet soaps and detergents. Both substances have antioxidant and antibacterial and also antiviral properties (Koroch et al., 2007).

Geraniol as a component of Citrus volatile was reported with very good free radical scavenging activity (Choi et al. 2000). The protective effect of myrcene and other two monoterpenes viz. eucalyptol and linalool were studied against t-butyl hydroperoxide induced genotoxicity in in bacteria and cultured human cells (Mitic-Culafic et al. 2009). The linalool and myrcene strongly suppressed t-BOOH induced mutagenesis. The results indicate that linalool, eucalyptol and myrcene have substantial protective effect against oxidant induced genotoxicity, which is predominately mediated by their radical scavenging activity.
6. **In vivo and in vitro effect of geraniol**

The structure of geraniol was shown Figure 2. The antihyperglycemic activity of geraniol was assessed through OGTT.

**Figure 2: Structure of geraniol**

```
\[
\text{OH}
```

6.1 **Oral Glucose Tolerance Test for geraniol**

The antihyperglycemic effect of geraniol, a major component present in *C. citratus* essential oil, was evaluated through OGTT. The results are shown in Figure 3.

**Figure 3. Oral Glucose Tolerance Test for geraniol**

![Graph showing Oral Glucose Tolerance Test for geraniol with different concentrations and control groups.](image-url)
The geraniol showed dose dependent antihyperglycemic activity in OGTT. At a concentration of 5 mg/kg, geraniol showed moderate blood glucose lowering effect as compared to glibenclamide control. At an increased concentration of 10 mg/kg, geraniol demonstrated very good antihyperglycemic activity. The blood lowering effect of geraniol at increased concentration was almost equally comparable to that of glibenclamide control. To evaluate mechanism for antihyperglycemic action, the *in vitro* insulin secretagogue action of geraniol was assessed further.

### 6.2 *In vitro* dose dependent effect of geraniol on insulin secretion

The isolated islets were treated with gradually increasing concentrations of geraniol in presence of stimulatory glucose concentration (11.8 mM). The results are shown in Figure 4. Insulin secretion in presence of 11.8 mM glucose was considered as negative control while 10 µg/ml of glibenclamide (a commercially used sulfonylurea drug) in presence of 11.8 mM glucose was used as positive control. Insulin secretion in each test was expressed in terms of µIU/10 islets/60 min.

**Figure 4. Dose dependent effect of geraniol on insulin secretion**

![](figure4.png)

Results are mean ± S.D.; n=6. *p<0.05 and **p<0.005 significant from 11.8 mM glucose control.
In the *in vitro* study on isolated islets, geraniol showed dose dependent insulin secretion stimulatory effect up to 25 µg/ml reaction mixture. The insulin stimulation at 50 µg/ml reaction mixture i.e. 134.20 µIU is higher as compared to that of glibenclamide which shows 115.93. However, geraniol requires almost 5 times higher concentration as compared to glibenclamide to show comparable insulin secretion. Hence, the results show that geraniol possess good insulin secretagogue action but is less potent as compared to the glibenclamide.

7. **In vivo and in vitro effect of myrcene**

Myrcene, the another major component present in the *C. citratus* oil was also assessed for its antihyperglycemic action through oral glucose tolerance test which then followed by *in vitro* effect of myrcene on insulin stimulation. The structure of myrcene is shown in Figure 5.

**Figure 5. Structures of myrcene**

![Structure of myrcene](image)

7.1 **Oral Glucose Tolerance Test for myrcene**

The antihyperglycemic effect of myrcene, another major component present in *C. citratus* essential oil, was evaluated through OGTT. The results are shown in Figure 6. The diabetic rats receiving 0.1% DMSO as a vehicle are considered as negative control while that with glibenclamide (2.5 mg/kg body weight) are considered as a positive control.
Figure 6. Oral glucose tolerance test for myrcene

At a concentration of 5 mg/kg body weight myrcene does not showed detectable antihyperglycemic activity. However, at an increased concentration to 10 mg/kg body weight, myrcene demonstrated good antihyperglycemic activity as compared to diabetic control. The antihyperglycemic activity of myrcene even at 10 mg/kg body weight concentration is not as potent as that of the glibenclamide (2.5 mg/kg body weight) which showed very good antihyperglycemic action.

The in vitro insulin secretagogue action of myrcene on isolated islets was further evaluated.

### 7.2  *In vitro* dose dependent effect of myrcene on insulin secretion

The isolated islets were challenged with gradually increasing concentrations of myrcene in presence of stimulatory glucose concentration (11.8 mM). Four concentrations of myrcene (6.25, 12.5, 25 and 50 µg/ml reaction mixture) were used. The results are shown in Figure 7. Insulin secretion in presence of 11.8 mM glucose was considered as negative control while 10 µg/ml of glibenclamide (a commercially used
sulfonylurea drug) in presence of 11.8 mM glucose was used as positive control. Insulin secretion in each test was expressed in terms of µIU/10 islets/60 min.

Figure 7. Dose dependent effect of myrcene on insulin secretion

Results are mean ± S.D.; n=6. *p<0.01 and **p<0.001 significant from 11.8 mM glucose control.

In the in vitro study on isolated islets, myrcene showed dose dependent insulin secretagogue action as compared to 11.8 mM glucose control. However, the insulin secretagogue activity of myrcene even at a higher concentration of 50 µg /ml reaction mixture was less potent than insulin secretagogue action of glibenclamide in terms of concentration/ml. The myrcene at a concentration of 50 µg/ml reaction mixture shows 100.70 µIU as compared to glibenclamide (10 µl/ml) which shows 115.93 µIU/10 islets/60 min. Hence, the myrcene showed good insulin secretagogue action but less potent than that of glibenclamide in given experimental conditions. Though, the myrcene showed good insulin secretagogue action in vitro, its antihyperglycemic action is not pronounced as that of geraniol or glibenclamide.
8. Conclusion

In conclusion, *C. citratus* essential oil and its components as geraniol and myrcene showed good antihyperglycemic action. The whole essential oil, geraniol and myrcene showed insulin secretagogue action. However among the three, geraniol demonstrated most potent insulin secretagogue action both in the *in vivo* and *in vitro* studies. Hence, *C. citratus* essential oil and its components as geraniol and myrcene can be used in treatment of diabetes mellitus. Hence, use of lemon grass on a regular basis by diabetic patients can be beneficial in lowering the blood glucose and potentiates other therapies used in diabetes treatment.

As stated earlier, while some plants such as *Mamordica charantia* or *Eugenia jambolana* possess very strong antihyperglycemic action and could lead to hypoglycemia if followed with other allopathic therapies. Plants such as lemon grass would not show such an action at the concentration used as teas. Moreover, it would definitely augment the other allopathic therapies used. This is very potential in that it would lower the dose of the synthetic components used as anti diabetic and thereby minimizes or lower their side effects leading to a more effective therapy. Needless to say a proper monitoring of the blood glucose is always going to be very essential for a correct and glycemic control.

9. References


III. Assessment of eventual toxic, hypnotic and anxiolytic effects on humans. *Journal of Ethnopharmacology* 17: 75-83.


