Chapter II

Phytochemical Constituents Analysis, Evaluation of Effective Dose and Proteome Analysis in *Cynodon dactylon* (L.) pers.
CHAPTER – 2

PHYTOCHEMICAL CONSTITUENTS ANALYSIS, EVALUATION OF EFFECTIVE DOSE AND PROTEOME ANALYSIS IN

*Cynodon dactylon* (L.) Pers.

2.1. INTRODUCTION

Herbal products were being the effective source of both traditional and modern medicines which are used extensively to treat several medical problems. Nature has been a source of medicinal agents since times immemorial. India is rich in biodiversity, which comprises the indigenous knowledge of traditional healers. In India, throughout its long history, has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of various diseases. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Chemical studies of Indian medicinal plants afford an important material for the detection and development of new drugs of natural origin. In the recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju et al., 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin et al., 1985). Use of plants as traditional health remedies is very popular and important for 80% of the world’s population in India, African, Asian, Latin America and Middle Eastern Countries. Their use is reported to have minimal side effects (Bibitha et al., 2002; Maghrani et al., 2005). Hence, more studies are pertaining to the use of the plant as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu et al., 1999). In recent years, pharmaceutical companies are spent considerable time and money in developing therapeutics based upon natural products extracted from plants (Ben et al., 2007; Coruh et al., 2007).
Herbs are extensively studied for their large therapeutical interests and benefits. Their complex mixture of bioactive compounds covers a large variety of demands for the human health. In the present study focuses the screening of phytochemical in C. dactylon and its pharmacological activities in diabetic rats.

2.2. MATERIALS AND METHODS

2.2.1. Collection and Authentication of Plant

The fresh leaves of C. dactylon were collected from PRIST University, Thanjavur, Tamilnadu. The plants were taxonomically identified and authenticated by Rev Dr S John Britto SJ, Director, The Rapinat Herbarium, St Joseph’s College (Autonomous), Tiruchirappalli, Tamil Nadu, India. The voucher specimen’s number is RHCD02.

2.2.2. Aqueous Extract Preparation

Fresh plant of C. dactylon was cleaned and shade dried. The dried plant was pulverized by an electrical blender and passed through 20 µ mesh sieve. The powdered plant up to 450 g was extracted with boiling water for 10 h. The resulted extracts were filtered and concentrated by lyophilizer. The yield of extract was 13.5 % (w/w) in terms of dried starting material (Chopra et al., 1992).

2.2.3. Ethanolic Extract Preparation

Fresh plant of C. dactylon was cleaned and shade dried. The dried plant was pulverized by an electrical blender and passed through 20 µ mesh sieve. The extraction was carried out by mixing the powdered (550 g) leaves with 1:2 (w/v) in 70% ethanol (v/v) for 2 days. The resulted extract was filtered and concentrated by rotary evaporator under reduced pressure and low temperature. The yield of extract was 12.2 % (w/w) in terms of dried starting material (Chopra et al., 1992).
2.2.4. Assessment of Hypoglycemic Activity in Normal Healthy Rats

Eight-week-old 130–150 g male albino wistar rats (Rat: *Rattus norvegicus*) \((n=24)\) were housed in PRIST University animal centre and were received normal rat feed and *ad libitum* in a constant environment (room temperature \((22 ± 2 °C)\), room humidity \((55 ± 4 \%)\) with a 12-hour light and dark cycle. The animals were kept under observation for two week prior to the start of experiment (acclimatization). All procedures were approved by the Institutional Animal Ethics Committee (IAEC) in PRIST University (IAEC No.: 743/03/abc/CPCSEA dt 3.3.03 - approval no.: PhD2/2009-2010).

Forty two animals of eight week old normal healthy male rats were fasted overnight. The fasted animals were divided equally into seven groups of six rats each. Pretreatment FBG levels of each group were evaluated. Group 1 served as untreated control given vehicle (water only), whereas the other three groups 2, 3 and 4 were given lyophilized aqueous extract suspended in distilled water orally in doses of 250, 450 and 650 mg/kg bw, respectively. The other three groups 5, 6 and 7 were given ethanolic extract suspended in distilled water orally in doses of 250, 450 and 650 mg/kg bw, respectively. Blood samples were collected from the tail vein after 4 hours and the percentage change in blood glucose were calculated for each group (Glucose estimation kit).

2.2.5. Assessment of Anti-Diabetic Activity by Glucose Tolerance in Diabetic Rats

The anti-diabetic effects of aqueous and ethanolic extract were assessed in diabetic rats using a glucose tolerance test. Albino rats were made diabetic by a single intraperitoneal injection (i.p.) of alloxan, dissolved in normal saline (0.9%) at a dose of 150 mg/kg of body weight (Uma *et al.*, 2006). They blood glucose higher than 200 mg/dl after three days were considered as being diabetic in the fasting state. The overnight fasted rats were divided into seven groups (8, 9, 10, 11, 12, 13 and 14) of six rats each. Pre-treatment FBG levels of each group were evaluated. Group 8 (diabetic control) received vehicle (water only) whereas the other three groups such as 9, 10 and
11 doses of 250, 450 and 650 mg/kg bw of aqueous extract were given orally. The other three groups 12, 13 and 14 were given ethanolic extract suspended in distilled water orally in doses of 250, 450 and 650 mg/kg bw, respectively. Ten grams per kilogram bw glucose solution was given to all the groups and their BGL were estimated after 1 and 2 h of glucose administration (Glucose estimation kit).

2.2.6. Assessment of Antidiabetic Potential for Plant Extract in Diabetic Rats

Six rats were randomly selected as control group \((n=6)\) and received a single intraperitoneal injection of 0.9 % saline only (group I). The other 18 rats were fasted for 24 hours prior to the induction of diabetes. Albino rats were made diabetic by a single intraperitoneal injection (i.p.) of alloxan, dissolved in normal saline (0.9 %) at a dose of 150 mg/kg of body weight. All 18 rats with blood glucose higher than 200 mg/dl after three days were considered as being diabetic in the fasting state. All studies were carried out after three days of alloxan injection. All rats were divided into three groups. The groups were divided as follows: group II, untreated diabetic rats (alloxan induced diabetic rats DM, \(n=6\)); groups III, treated diabetic rats with aqueous \(C.\ dactylon\) leaves extract (ACDLE; 450 mg/kg/bw) at 24 h intervals for 15 days and group IV: treated diabetic rats with ethanolic \(C.\ dactylon\) leaves extract (ECDLE; 450 mg/kg/bw) at 24 h intervals for 15 days. At the end of the experimental period, the animals were fasted overnight (12 hours) and then collected blood with heparin (10 IU) from tail vein for this study. The blood sample were centrifuged at 6000 rpm at 4 °C for 10 min and collected plasma and then rapidly stored at −80 °C.

2.2.7. LD50

Two groups of rats (6 animals per group) were administered orally by a single dose of and 10 times of the effective dose of the aqueous and ethanolic extract of \(C.\ dactylon\). The rats were observed for gross behavioural, neurologic, autonomic and toxic effect at regular intervals. Food consumption, faeces and urine were also examined at 2 h and then at 6 h intervals for 24 h.
2.2.8. Estimation of Glucose (AGAPPE Diagnostics Kit)

10 µl of plasma sample mixed with 1000 µl of enzyme reagent and incubated for 10 minutes at 37 °C. At the same time blank and standard solution was prepared. The absorbance of sample against reagent blank was read at 505 nm. The activity was calculated by using the formula. Glucose (mg/ml) = Abs. sample/Abs. standard × conc. of standard. The values are expressed in mg/dl.

2.2.9. GC-MS Analysis

Aqueous and ethanolic extract of *C. dactylon* were analyzed by gas chromatography equipped with mass spectrometry (GC-MS-QP2010-Shimadzu). The chromatographic conditions were as follows: Column: DB-% ms (length 30.0 m, Diameter 0.25 mm, Film thickness 0.25 µm). The 1µl plant extracts (aqueous and ethanolic extract separately) were injected into the GC-MS in split less mode at 200 °C. The column oven temperature was held at 45 °C for 1 minute, then programmed at 10 different rates up to 280 °C and held for 18 minutes. Helium carrier gas was maintained at a flow rate of 1.4 ml/min.

2.2.10. Two-Dimensional Gel Electrophoresis

The powder (0.3 g) was re-suspended in 2 ml of plant extraction buffer containing 20mm Tris pH 8.0, 5mm EDTA, 0.05 % SDS. Then ice-cold acetone-10 % TCA (Sigma) added to supernatant and sonicated for 1 min. The homogenate was kept for precipitation overnight at -20 °C. After centrifugation at 10000 rpm for 15 min at 4 °C, the supernatant was removed by decanting immediately and the pellet was rinsed twice in ice-cold acetone for four times. The pellet was then air-dried, re-suspended in a lysis buffer containing (85 mM Tris pH 6.8, 2 % SDS). Iso-electric focusing (IEF) gels (O'Farrell, 1975) were made in glass tubing (160 × 3 mm inside diameter) and contained 10.3 g urea, 7.125 ml distilled water, 2.44 ml acrylamide (28.38% acrylamide, 1.62 % bis-acrylamide), 0.750 ml carrier ampholytes 3/10, 0.375 ml NP-40, 34.625 µl ammonium persulphate (10 %) and 12.5 µl TEMED. After half an hour’s polymerisation, a pre-run for focusing the ampholytes was performed by loading 30 µl
lysis solution (9.8 M urea, 2 % NP-40 (10 % in distilled water), 2 % carrier ampholytes 8/10, 25 mM DTT) and over 30 µl overlay solution (8Murea, 1 % carrier ampholytes 8/10, 5 % NP-40, 25 mM DTT). Upper running buffer (20 mM NaOH) was degassed for 10 min, but the lower one (10 mM H$_3$PO$_4$) was not. The electrophoretic conditions of the rod gels during the IEF were 200 V for 15 minutes, 300 V for 30 minutes and 400 V for 1 h. The solution was removed from the upper tank. Meanwhile, the sample (350 µg) (Bradford, 1976) was prepared by adding lysis solution in a 1:2 proportion and heating for 2 min at 100 °C. After loading, 30 µl of overlay solution was added above every sample, which was then run at 400 V for 16 h. After focusing, IEF gels were maintained in equilibrating buffer (6 M urea, 75 mM Tris-HCl pH 8.8, 29.3 % glycerol, 2 % SDS, 0.002 % bromophenol blue) for 30 minutes. Then, the IEF rod gels were immediately applied to an SDS-polyacrylamide gel that contained 12 % acrylamide, but the stacking gel was replaced by IEC rod gels fixed to the SDS-PAGE gel with an agarose solution (1 % agarose, 0.002 % bromophenol blue in the first equilibrating buffer). The analytical gels were stained with colloidal blue.

2.2.11. Statistical Analysis

The data are expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA). The results were considered statistically significant if the $P$ values were 0.05 or less.

2.3. RESULTS

2.3.1. Assessment of Hypoglycemic Activity in Normal Healthy Rats

Results of the effect of aqueous and ethanolic extract of *C. dactylon* on blood glucose levels of normal rats are presented in table 2.1. All the three doses of the extract produced significant hypoglycemic effect after 4 h oral administration. The animals treated with dose of 250 and 650 mg/kg produce a fall of 19.2 %, 22.9 % for AET and 18 %, 23.9 % EET, after 4 h of extract administration. However, it was more marked as 27.8 % for AET and 29.2 % for EET in the animals treated with a dose of 450 mg/kg bw after 4 h of administration. The hypoglycemic effect was moderate after 2 h of the extract administration with all the three doses (8–15 %).
Table 2.1 – Hypoglycemic impact of graded doses of aqueous and ethanolic extract of C. dactylon in normal rats and Impact of oral administration of the aqueous and ethanolic extract of C. dactylon on FBG. Values are expressed mean ± S.E.M. of six animals.

**Abbreviations:** DW: Distilled Water; FBG: Fasting Blood Glucose; BGL: Blood Glucose Level; GTT: Glucose Tolerance Test C: Control; AET: Aqueous Extract Treated; EET: Ethanolic Extract Treated; DC: Diabetic Control.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Dose (mg/kg of Body Weight)</th>
<th>FBG (mg/dl)</th>
<th>After Treatment (mg/dl)</th>
<th>Hypo-Glycemic Activity</th>
<th>FBG</th>
<th>2 h</th>
<th>4 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DW</td>
<td>DW</td>
<td>84.6±2.7</td>
<td>80.1±2.4</td>
<td>82.7±3.5</td>
<td></td>
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<tr>
<td>2.</td>
<td>AET 250</td>
<td>81.5±6.5</td>
<td>72.1±3.8</td>
<td>65.8±4.2</td>
<td>19.2</td>
<td>11.5</td>
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<tr>
<td>3.</td>
<td>AET 450</td>
<td>78.3±4.5</td>
<td>66.8±4.8</td>
<td>55.4±4.5</td>
<td>29.2</td>
<td>14.6</td>
<td></td>
<td></td>
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<tr>
<td>4.</td>
<td>AET 650</td>
<td>78.8±7.5</td>
<td>69.5±3.8</td>
<td>60.7±4.5</td>
<td>22.9</td>
<td>11.3</td>
<td></td>
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<tr>
<td>5.</td>
<td>EET 250</td>
<td>80.5±4.8</td>
<td>73.8±4.2</td>
<td>66.0±2.2</td>
<td>18.0</td>
<td>8.3</td>
<td></td>
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<tr>
<td>6.</td>
<td>EET 450</td>
<td>79.3±3.8</td>
<td>70.5±4.6</td>
<td>57.6±3.5</td>
<td>27.3</td>
<td>11.0</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>7.</td>
<td>EET 650</td>
<td>80.1±5.8</td>
<td>72.2±3.1</td>
<td>60.9±2.9</td>
<td>23.9</td>
<td>9.8</td>
<td></td>
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<td></td>
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</table>
2.3.2. Assessment of Anti-Diabetic Activity on Glucose Tolerance in Diabetic Rats

In order to choose the optimum dose for the diabetic animals, different doses of aqueous and ethanolic extract (250, 450 and 650 mg/kg bw) were evaluated on glucose tolerance in diabetic rats. Table 2.1 depicts the effect of the above mentioned doses of \textit{C. dactylon} aqueous and ethanolic extract on glucose tolerance up to 2 h in diabetic rats. A reduction of 10\% of AET and 8.2\% of EET in BGL were observed within 2 h of GTT by the dose of 250 mg/kg bw and this fall increased further to 19.6\% of AET and 18.6\% of EET with 450 mg/kg bw of the extract. Anti-diabetic effect of graded doses of aqueous and ethanolic extract of \textit{C. dactylon} on glucose tolerance in diabetic rats \((P < 0.05)\). Moreover, the higher dose of 650 mg/kg bw had reduction of 12.1\% of AET and 15\% of EET in BGL were observed within 2 h of GTT. It therefore appears that 450 mg/kg bw of the aqueous and ethanolic extract of \textit{C. dactylon} is the most effective dose on GTT of alloxan-induced diabetic rats and hence it was selected as a effective dose for evaluation of anti-diabetic activity in alloxan-induced diabetic rats.

2.3.3. Assessment of Antidiabetic Potential for Plant Extract in Diabetic Rats

The impact of repeated oral administration of \textit{C. dactylon} leaves aqueous and ethanolic extract on fasted alloxan-induced rats is shown in figure 2.1. FBG levels remain practically the same before and after the treatment with vehicle (water only) in case of control rats. Whereas, in diabetic rats the FBG rises gradually in 2 weeks. Moreover, the 2-week treatment with the most effective dose (450 mg/kg bw) of the extract decreases FBG significantly from 240 mg/dl to 130 mg/dl for FBG 1h 2h 3h

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Effect of aqueous and ethanolic extract of \textit{C. dactylon} leaves on blood glucose levels in alloxan induced-diabetic rat after feeding glucose (10 g/kg). Values are expressed mean ± SEM of six animals.}
\end{figure}
AET and 230 mg/dl to 155 mg/dl for EET indicating thereby a fall of 45.8 % for AET and 32.6 % for EET in BGL. This sharp fall of 45.8 % for AET and 32.6 % for EET in BGL is a clear evidence of significant anti-diabetic effect of *C. dactylon*.

2.3.4. LD50

Experiment was carried out on normal healthy rats. The behaviour of the treated rats appeared normal. No toxic effect was reported up to 5 and 10 times of the effective dose of the aqueous and ethanolic extract and there were no death in any of these groups.

2.3.5. GC-MS Analysis of *C. dactylon* Extract

The GC-MS analysis of *C. dactylon* extract detected molecular peaks, with typical retention time of analyzed components, which are depicted in total ions chromatogram (TIC) obtained from MS analysis are shown in figure 2.2. We have reported the constituents of aqueous and ethanolic extract of *C. dactylon* and its chemical structure which are presented in figure 2.3a, 2.3b and table 2.2, 2.3. The GC-MS analysis of plant extract revealed the presence of...
7 major compounds in aqueous extract i.e. Phenylmethanol (R; 7.67), 2-Propenoic acid (Cinnamic acid) (R; 11.98), Sesquiterpene (R; 15.45), 2-methoxy-4-prop-2-enylphenyl acetate (R; 16.1), 4',5,7-Trihydroxyisoflavone (R; 16.27), Procyanidin (R; 16.9) and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (R; 17.6).

The GC-MS analysis of plant extract revealed the presence of 6 major compounds in ethanolic extract i.e. 2-Propenoic acid (Cinnamic acid) (R; 11.98), β-3,7-dimethyl-1,3,6-octatriene (R; 12.14), 3-(3,4-Dihydroxyphenyl 2-propenoic acid (R; 12.96), 2-isopropyl-5-methyl cyclohexyl] ester (R; 13.25), 4-Ethenyl-2-methoxyphenol (R; 13.3) and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (R; 17.6). 2-Propenoic acid and tetramethyl-2-hexadecen-1-ol are present in both aqueous and ethanolic extract of *C. dactylon*. 
Table 2.2 – Compounds present in aqueous extract of *C. dactylon* leaves. *t*R; Retention Time.

<table>
<thead>
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<th>S No.</th>
<th>Formula</th>
<th>Molecular weight (da)</th>
<th>Component Name</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>C\textsubscript{7}H\textsubscript{8}O</td>
<td>108.14</td>
<td>Phenylmethanol</td>
</tr>
<tr>
<td>2</td>
<td>C\textsubscript{9}H\textsubscript{8}O\textsubscript{2}</td>
<td>148.16</td>
<td>2-Propenoic acid (Cinnamic acid)</td>
</tr>
<tr>
<td>3</td>
<td>C\textsubscript{15}H\textsubscript{24}</td>
<td>204.11</td>
<td>Sesquiterpene</td>
</tr>
<tr>
<td>4</td>
<td>C\textsubscript{12}H\textsubscript{14}O\textsubscript{3}</td>
<td>206.23</td>
<td>2-methoxy-4-prop-2-enylphenyl) acetate</td>
</tr>
<tr>
<td>5</td>
<td>C\textsubscript{15}H\textsubscript{10}O\textsubscript{5}</td>
<td>270.24</td>
<td>4’,5,7-Trihydroxyisoflavone</td>
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<tr>
<td>6</td>
<td>C\textsubscript{15}H\textsubscript{11}O\textsubscript{6}</td>
<td>287.12</td>
<td>Procyanidin</td>
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<tr>
<td>7</td>
<td>C\textsubscript{20}H\textsubscript{40}O</td>
<td>296.54</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
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</tbody>
</table>

Table 2.3 – Compounds present in ethanolic extract of *C. dactylon* leaves. *t*R; Retention Time.

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Formula</th>
<th>Molecular weight (da)</th>
<th>Component Name</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>C\textsubscript{9}H\textsubscript{8}O\textsubscript{2}</td>
<td>148.16</td>
<td>2-Propenoic acid (Cinnamic acid)</td>
</tr>
<tr>
<td>2</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>136.24</td>
<td>â-3,7-dimethyl-1,3,6-octatriene</td>
</tr>
<tr>
<td>3</td>
<td>C\textsubscript{9}H\textsubscript{8}O\textsubscript{4}</td>
<td>180.16</td>
<td>3-(3,4-Dihydroxyphenyl 2-propenoic acid</td>
</tr>
<tr>
<td>4</td>
<td>C\textsubscript{12}H\textsubscript{22}O\textsubscript{2}</td>
<td>198.30</td>
<td>2-isopropyl-5-methylcyclohexyl] ester</td>
</tr>
<tr>
<td>5</td>
<td>C\textsubscript{9}H\textsubscript{10}O\textsubscript{2}</td>
<td>150.18</td>
<td>4-Ethenyl-2-methoxyphenol</td>
</tr>
<tr>
<td>6</td>
<td>C\textsubscript{20}H\textsubscript{40}O</td>
<td>296.54</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
</tr>
</tbody>
</table>
Figure 2.3a – GC-MS analysis of scanned peak of individual components with retention time and Chemical structure of identified components from aqueous extract of C. dactylon.

Figure 2.3b – GC-MS analysis of scanned peak of individual components with retention time and Chemical structure of identified components from ethanolic extract of C. dactylon.
2.3.6. Proteome Analysis of *C. dactylon* Leaves

The 2-DE analysis of proteins pattern from *C. dactylon* leaves showed with relative molecular weight marker were between 10 and 170 kDa as indicated in figure 2.4. Of note, salt, carbohydrate, lipid and other secondary metabolic components were found to interfere with the iso-electric focusing in the first dimension. Thus, a two-step method (Ice-cold acetone/TCA precipitation and cold acetone wash) was used to remove these interfere components before samples were subjected to 2-DE. Iso-electric points were between 3 and 10 using range pH 3–10 pH ampholyte (17 cm) and most of them were in the range of 3.5–7 pH. Totally about 95 protein spots could be visualized on the 2-DE maps by using a web based gel viewing and annotation system (http://www.gelscape.ualberta.ca). The majority of these spots matched in three individually processed gels and they were very similar in intensity, indicating this method is highly reproducible.

*Figure 2.4 Two-dimensional electrophoresis patterns of proteins from *C. dactylon* leaves (pH 3–10). (A) SDS-PAGE; (B) 2-DE and (C) 3D view of protein spots.*
2.4. DISCUSSION

The findings of this study indicate that an aqueous and ethanolic extract of *C. dactylon* had a significant hypoglycemic effect in normal rats, up to 4 h. The effect was dose-dependent up to 450 mg/kg bw. However, the response decreased at higher dose of 650 mg/kg bw dose. Such a phenomenon of less hypoglycemic response at higher doses is common in indigenous plants and has already been observed in *Aegle marmelose* (Rao *et al*., 1995) and *Murraya koenigii* (Kesari *et al*., 2005).

The GTT studies of the diabetic animals reveal a maximum fall of 19.6 % of AET and 18.6 % of EET in 2 h by the dose of 450 mg/kg bw whereas, the doses of 250 and 650 mg/kg bw produced almost similar fall of about 10–15 % only in BGL. The GTT studies also confirm 450 mg/kg bw to be the most effective dose as found in the case of normal animals. This dose was therefore, selected for further studies in the case of diabetic animals and a fall of 45.8 % for AET and 32.6 % for EET were observed in FBG after 14 days of treatment. The administration of *C. dactylon* leaves extract to alloxan diabetic rats reduced blood glucose levels, is in accordance with previous studies (Ravi *et al*., 2004). These effects may be attributed to the insulin mimicking effect of plant extract (Bolkent *et al*., 2005).

The GC-MS analysis of plant extract revealed the presence of 7 major compounds in aqueous extract and 6 major compounds in ethanolic extract. 2-Propenoic acid and tetra methyl-2-hexadecen-1-ol are present in both aqueous and ethanolic extract of *C. dactylon*. Based on our GC-MS result, we could predict that, 2-propenoic acid and tetra methyl-2-hexadecen-1-ol could be having anti-diabetic activity. Phytochemical investigation of *C. dactylon* reveals the presences of flavonoids and sterols (Patil *et al*., 2005). These principles are known to be bioactive for the management of diabetes (Rhemann and Zaman, 1989). It is well known that certain flavonoids exhibit hypoglycemic activity (Ahmad *et al*., 2000) and is also known for their ability of beta cell regeneration of pancreas (Chakravarti *et al*., 1981). Sterols have also shown to decrease blood sugar in experimental animal models (Suba *et al*., 2004). Thus, the significant antidiabetic effect of aqueous and ethanolic extract of *C. dactylon*
may be due to the presence of more than one antihyperglycemic principle and their synergistic properties.

Two-dimensional electrophoresis analysis of proteins extracted from *C. dactylon* was performed using pH range 3–10. It implied the potential application of the comparative proteomics study of *C. dactylon* in understanding the protein expression and their function.

From this study we can conclusively state that, *C. dactylon* aqueous and ethanolic extract has shown remarkable effects on blood glucose level and marked improvement on diabetes. The comparative analyses of both the extract were given similar kind of result. The extract seems to have no toxicity as no death is reported up to 10 times of effective dose. These results suggest that the product of *C. dactylon* may provide a new therapeutic avenue against diabetes and diabetes-related complications—a global burden. Moreover, natural health products of vegetable origin were clearly indicated as a promising avenue for the prevention of chronic diseases (Punitha *et al.*, 2005), as raised notably by a panel of experts that convened in Haddad *et al.* wide-spread research is currently taking place in China, India and other countries too in order to explore new traditional Chinese, Indian and Western medicines that will prevent and treat diabetes mellitus and its chronic complications (Haddad *et al.*, 2005).