3.0 Need for Standardization of Herbal Drug

Management of Diabetes mellitus (DM) has been raised as a huge threat among medical fraternity as it endorses a significant disruption in carbohydrate, fat & protein metabolism. The practice of complementary & alternative therapies has been steadily increasing throughout the world. World Health Organisation (WHO) in its resolution AFR/RC50/R3 (2000) (1) has recommended the practise of natural based alternative therapy in research & clinical applications, where the resources are found to be abundant (2). At present global setup, nearly 25% of pharmaceutical drugs are derived from natural based products scales up the annual market about US$ 60 billion (3, 4). In recent ages numerous natural or its derived lead compounds with desired pharmacological activity have been in practise to combat DM mediated effects. At present, even though numerous anti-diabetic herbal products are in practice, less evidence sighting its safety & efficiency has been reported.

In some cases, misinterpretation of raw materials, herbicide & pesticide contamination, heavy metal load guided to the numerous fatal side effects in human population. Clinical report highlighted the existence of heavy metals in Chinese herbal medicine and further ended up with Pre-comatose state, persistent hypoglycaemia in 56 year old patient for Type 2 Diabetes mellitus treatment (5). Recent study in Asia suggest that, out of 260 patented medicines nearly 25% of materials are recorded with high levels of heavy metal contents and 7% of drugs are measured with adulterants (6). World Health Organization (WHO) also urged the need for essential guidelines to certify the quality of herbal based products by involving appropriate parameters & standard protocols (7). According to Chawla et al., herbal products must follow the safety measures such as detection of adulterants & heavy metal contamination, appropriate toxicity studies and
improvement of standard assay markers to attain the maximum drug efficacy (8). For the enhancement of herbal products, World Health Assembly in its resolution WHA31.33 (1978), WHA40.33 (1987) & WHA42.43 (1989) has endorsed the application of modern sophisticated techniques to ensure the quality of medicinal plant products (9) (Fig.29). In current chapter ADPHF6 polyherbal formulation, was screened for secondary metabolites/phytochemical evaluation using GC-MS analysis.

Fig 29: Standardization of Herbal Drugs (10)

Though, compounds or secondary metabolites derived from natural sources play numerous role in a biological system, less emphasis has been itemised in findings the trace elements for chronic endocrine disorder. In human biological system, trace elements conceal less than 0.01% of body mass (11, 12). Essential elements such as calcium (Ca), potassium
(K), magnesium (Mg), aluminium (Al) act as catalysts in myriads of enzymatic reactions & it also regulates basic physiological processes (13, 14). Interestingly, the trace elements play an essential role in improving impaired glucose tolerance & in reducing obesity. Reports also cite that zinc (Zn), manganese (Mn), magnesium (Mg) involves in activation of insulin receptor sites, increases the insulin sensitivity, inhibits tissue peroxidation’s, act as a cofactor in glucose metabolism etc., which eventually leads to enhancement of insulin action (15). However, administration of these trace elements as monotherapy will hardly make progress during diabetic condition (16). A combination of more than one plant is expected to provide best synergistic activity (17) and also various agonistic/antagonistic actions (18). In our present chapter, polyherbal formulation ADPHF6 has been subjected for trace element & heavy metal detection by involving Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), SEM-EDAX & Laser Induced Breakdown Spectroscopy (LIBS) analysis. Further ADPHF6 was also studied for physical & chemical changes by involving Thermogravimetric & differential scanning calorimetry analysis.

3.1 Materials & Methods

3.1.1 GC-MS (Gas Chromatography & Mass Spectrometry) analysis

3.1.1.1 Sample preparation & Identification of Components

About 1 g of lyophilized ADPHF6 polyherbal formulation was suspended in 10 ml of different solvents such as Ethanol, Petroleum ether, Hexane (MerckMillipore, India). The ADPHF6 samples was then sonicated for 2 h and further kept for 12 h incubation at room temperature in a screw cap vial (19). After instrumental analysis, interpretation on mass spectrum of GC-MS was carried out using the database of in-built library, NIST 8 (National Institute of Standards and Technology). The compound name, RT value, percentage peak area and molecular weight of the components were determined.
3.1.1.2 Instrumentation

The GC-MS based phytochemical screening for ethanol extract of ADPHF6 was performed using JEOL GC MATE II GCMS, interfaced to a Mass Spectrometer (Quadruple Double Focusing Mass Analyser) equipped with HP-5-MS capillary standard column (Length: 30.0 m, Diameter: 0.25 mm, Film thickness: 0.25 μm) and also with an AOC-20i auto injector. For GCMS detection, an electron ionization energy system with ionization energy of 70 eV was used. Helium gas (High Pure i.e. 99.999%) was used as the carrier gas at a constant flow rate of 1.0 ml/min and an injection volume of 1 ml was employed. Injector temperature was set at 250⁰C and the ion-source temperature was at 250⁰C. The oven temperature was programmed to 50 to 250 @ 10 deg / min. Mass spectra were taken at 70 eV with scan interval of 0.50 s with scan range of 40-1000 m/z. Total GC running time was 48 min. Data handling was made through JEOL software and matched with NIST library

3.1.2 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Analysis

3.1.2.1 Reagents required

1. Supra-pure concentrated nitric acid: 69%.
2. Sterile Milli-Q water.
3. NIST traceable Standards [Arsenic (As), Cadmium (Cd), Calcium (Ca), Chloride (Cl), Copper (Cu), Manganese (Mn), Mercury (Hg), Nickel (Ni), Phosphorous (P), Potassium (K), Selenium (Se), Tin (Sn), Zinc (Zn)].

3.1.2.2 Sample Preparation for Solid matrices

Freeze dried polyherbal formulation about 1.0 ± 0.1 g was well homogenized before suspending into microwave digestion vessel. About 5 ml of concentrated supra pure nitric
acid, 2 ml of 30% Hydrogen peroxide (H₂O₂) & 10 ml of Milli-Q water were added for free digestion.

3.1.2.3 Sample Digestion

Digestion vessel was loaded into microwave digester (Multi wave Pro, Anton Paar) & initial temperature: power was maintained at 0:400 watt for 15 minutes. Power was maintained at 400 watt for 10 mints until temperature reached 55°C. Once temperate reached 55°C, digestion vessels was allowed to cool; lid & walls of vessels were rinsed with Milli-Q water. Solutions were transferred into 25ml volumetric flasks, and diluted with Milli-Q water. The aliquot was filtered in an ash less filter paper and subjected for elemental analysis by ICP-OES (Agilent ICPOES720-ES, 725-ES, USA) with optimum conditions (Table 8).

<table>
<thead>
<tr>
<th>Table 8: ICP-OES Instrumental Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP-OES System</td>
</tr>
<tr>
<td>Optimised Parameter</td>
</tr>
<tr>
<td>RF Power</td>
</tr>
<tr>
<td>1.20 (Kw)</td>
</tr>
<tr>
<td>Plasma flow</td>
</tr>
<tr>
<td>15.0 (L/min)</td>
</tr>
<tr>
<td>Auxiliary flow</td>
</tr>
<tr>
<td>1.50 (L/min)</td>
</tr>
<tr>
<td>Nebulizer flow</td>
</tr>
<tr>
<td>0.75 L/min</td>
</tr>
<tr>
<td>Replicate reading time(s)</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Sample uptake delay (s)</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>Pump rate (rpm)</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>Rinse time (s)</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>
3.1.3 SEM - Energy Dispersive X-ray (SEM-EDAX) analysis

The lyophilized ADPHF6 sample was confirmed for minimal moisture (1-2%) content using a temperature probe and was further subjected to multi-elemental analysis at Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology Madras (IITM), Chennai. Initially, powder sample (size <10 mm) was converted into button shaped pellet and coated with gold material for better conductivity. High resolution morphological images were imaged with a scanning electron microscope (Quanta 200 FEG (SEM)). SEM was equipped & connected with Energy dispersive X-ray analytical system (Model EDAX) to detect the elemental composition of the polyherbal sample.

3.1.4 Laser Induced Breakdown Spectroscopy (LIBS)

3.1.4.1 Sample Preparation

The Uniformity of samples are prerequisite, in order to obtain the expected outcome of LIBS analysis. Freeze dried ADPHF6 sample was finely grinded to obtain the uniformity for spectral analysis (20). The lyophilized ADPHF6 powder was confirmed for minimal moisture (1-2%) content using a temperature probe and was reformed into pellets using a hydraulic press (KBr Press (manual), India) with 10 tons of pressure.

3.1.4.2 Instrumentation

LIBS instrumental setup comprises Q-switched Neodymium: Yttrium-Aluminium-Garnet (Nd: YAG) laser (Continuum Surellite III-10), stage carries sample (ADPHF6), multichannel spectrometer (Ocean optics LIBS 2000+) equipped with charged coupled device (CCD) detector (Fig. 30). At focal length of 25cm, quartz lens was focussed on the ADPHF6 sample and successively plasma appeared on the surface of the pellet. The emitted signal from plasma was collected and fed into four channelled spectrometer using optical fibre at
45°. The LIBS spectra were recorded at 10 Hz laser frequency and 40 mJ laser energy. Specific concentration of trace elements was measured by Boltzmann plot,

\[
\ln \frac{I_{ki}}{A_{ki} g_k} = -\frac{E_k}{k_B T} + \ln \frac{C_s \xi}{U_s(T)}
\]

Where \( k_B \) is the Boltzmann constant, \( \lambda \) is the wavelength of the transition, \( A_{ki} \) is the transition probability, \( g_k \) is degeneracy factor, \( I_{ki} \) represents the measured integral line intensity, \( C_s \) is the concentration of the emitting atomic species, \( U_s(T) \) is the partition function of that specie at plasma temperature (T), and \( F \) is an experimental parameter.
3.1.5 Thermogravimetric (TGA)

The ADPHF6 polyherbal formulation was measured for thermal degradation & stability changes using a Thermogravimetric analyser (TGA Q500, TA Instruments, USA) at Department of Chemistry, Indian Institute of Technology Madras (IITM), Chennai. TGA analysis was carried out with sample purge gas high pure nitrogen atmosphere by retaining a rate of 20°/min, heating rate of 10°C/min. ADPHF6 sample weighed of 6.9 mg was initially preheated and further allowed to above 900°C.

3.2 Results

3.2.1 GC-MS analysis

The phytochemicals present in ADPHF6 polyherbal formulation was identified by GC-MS analysis. GC-MS chromatogram of Ethanol & Hexane extract of ADPHF6 polyherbal formulation signifies the various phytochemical constituents as shown in Fig 4 & 5. The mass spectra of phytochemical compounds were identified by comparing with the NIST library data. GC-MS chromatogram (Figure 31) of the ADPHF6 ethanol fractions revealed nine bioactive compounds with the Peak area (%), Molecular weight & Retention time (RT) are illustrated in Table 9. In ADPHF6 ethanol fractions, 1,2-Benzenedicarboxylic acid mono (2-ethylhexyl) ester measured as maximum with 45.96 % peak area (RT 23.22) followed by 9, 12-Octadecadienoic acid ethyl ester with 13.06 % Peak Area (RT 19.43) (Fig. 32-41).
Fig 31: GC-MS Chromatogram of ADPHF6 Ethanol Fraction
Table 9: List of Compounds from GC-MS analysis of ADPHF6 Ethanol Fraction

<table>
<thead>
<tr>
<th>Phytochemical Compound</th>
<th>Retention Time (RT)</th>
<th>Molecular Formula</th>
<th>Mol. Weight</th>
<th>% Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Acetyl-17-(1,5-dimethyl),10,13 dimethylhexadecahydrocyclopenta(a)phenanthren-2-one</td>
<td>16.02</td>
<td>C₂₉H₄₈O₂</td>
<td>428.00</td>
<td>3.86</td>
</tr>
<tr>
<td>5,19 Cyclo-5a-andrsost-6-ene-3,17-dione</td>
<td>17.8</td>
<td>C₁₉H₂₆O₂</td>
<td>284.00</td>
<td>7.20</td>
</tr>
<tr>
<td>Carda-4,20(22)-dienolide,3-[(6-deoxy-3-O-methyl-a-L-mannopyranosyl)oxy]-14-hydroxy,[3a]</td>
<td>19.1</td>
<td>C₃₀H₄₄O₈</td>
<td>354.00</td>
<td>4.78</td>
</tr>
<tr>
<td>9,12-Octadecadienoic acid, ethyl ester</td>
<td>19.43</td>
<td>C₂₀H₃₆O₂</td>
<td>308.00</td>
<td>13.06</td>
</tr>
<tr>
<td>10-Hydroxy-5,7-dimethoxy-2,3-dimethyl-1,4-anthracenedione</td>
<td>19.72</td>
<td>C₁₈H₁₆O₅</td>
<td>312.00</td>
<td>3.45</td>
</tr>
<tr>
<td>4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol</td>
<td>20.43, 21.75</td>
<td>C₃₀H₅₂O</td>
<td>428.00</td>
<td>3.67</td>
</tr>
<tr>
<td>Benzophenone, 5-isopropyl-2-methyl</td>
<td>22.52</td>
<td>C₁₃H₁₀O</td>
<td>238.00</td>
<td>7.92</td>
</tr>
<tr>
<td>1,2-Benzenedicarboxylic acid mono(2-ethylhexyl) ester</td>
<td>23.22</td>
<td>C₁₆H₂₂O₄</td>
<td>279.00</td>
<td>45.96</td>
</tr>
</tbody>
</table>
Fig 32: 3-Acetyl-17-(1,5-dimethyl),10,13 dimethylhexadecahydrocyclopenta(a)phenanthren-2-one
Fig 33: Carda-4, 20 (22)-dienolide, 3-[6-deoxy-3-O-methyl-a-L-mannopyranosyl]oxy]-14-hydroxy, [3a]
Fig 34: 9, 12-Octadecadienoic acid, ethyl ester
Fig 35: 4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol
Fig 36: Benzophenone, 5-isopropyl-2-methyl
Fig 37: 1,2-Benzenedicarboxylic acid mono(2-ethylhexyl) ester
Fig 38: Ibogamine-18-carboxylic acid, 16, 17-didehydro-9, 17-dihydro-9,20-dihydroxy-12-methoxy-, methyl ester, [20S]
Fig 39: 10-Hydroxy-5, 7-dimethoxy-2,3-dimethyl-1,4-anthracenedione
Fig 40: 5,19 Cyclo-5a-androst-6-ene-3,17-dione
Fig 41: 4, 4a, 6b, 8a, 11, 11b-Octamethyl-docosahydropicen-3-ol

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Fig 42: GC-MS Chromatogram of ADPHF6 Hexane Fraction
Table 10: List of Compounds from GC-MS analysis of ADPHF6 Hexane Fraction

<table>
<thead>
<tr>
<th>Phytochemical Compound</th>
<th>RT</th>
<th>Molecular Formula</th>
<th>% Peak Area</th>
<th>Mol. Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclohexanone, 5-ethenyl-5-methyl-4-(1-methylethenyl)-2-(1-methylethylidene)-, cis</td>
<td>15.07</td>
<td>C$<em>{15}$H$</em>{22}$O</td>
<td>5.36</td>
<td>218.00</td>
</tr>
<tr>
<td>Ethanone,1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-4H-cyclohepta(b)furan-5yl)</td>
<td>17.17</td>
<td>C$<em>{14}$H$</em>{20}$O$_2$</td>
<td>4.63</td>
<td>205.00</td>
</tr>
<tr>
<td>Benzoic acid, 3-formyl</td>
<td>17.6</td>
<td>C$<em>{8}$H$</em>{6}$O$_3$</td>
<td>4.84</td>
<td>149.00</td>
</tr>
<tr>
<td>14,17-Octadecadienoic acid, methyl ester</td>
<td>18.85</td>
<td>C$<em>{19}$H$</em>{34}$O$_2$</td>
<td>8.10</td>
<td>294.00</td>
</tr>
<tr>
<td>Butylaldehyde, 4-benzyloxy-4-(2,2,-dimethyl-4-dioxolanyl)</td>
<td>23.47</td>
<td>C$<em>{16}$H$</em>{22}$O$_4$</td>
<td>77.06</td>
<td>277.00</td>
</tr>
</tbody>
</table>

The GC-MS chromatogram (Fig. 42) of the ADPHF6 hexane fractions, spiked five bioactive components with the Peak area (%), Molecular weight & retention time (Min) are illustrated in Table 10. In hexane fraction, Butylaldehyde, 4-benzyloxy-4-(2,2,-dimethyl-4-dioxolanyl) recorded as maximum with 77.06% Peak Area (RT 23.47) followed by 14-17 Octadecadienoic acid ethyl ester with 8.10 % Peak Area (RT 18.85) (Fig 43-47).
Fig 43: Cyclohexanone, 5-ethyl-5-methyl-4-(1-methylethenyl)-2-(1-methylethylidene), cis
Fig 44: Ethanone, 1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-4H-cyclohepta(b)fur-an-5-yl)
Fig 45: Butylaldehyde, 4-benzyloxy-4-(2,2-dimethyl-4-dioxolanyl)
Fig 46: 14, 17-Octadecadienoic acid, methyl ester
Fig 47: Benzoic acid, 3-formyl
3.2.2 ICP-OES Analysis

Trace element composition in ADPHF6 polyherbal formulation sample was determined by ICP-OES was summarised in Table 11. ICP-OES analysis revealed the concentration of heavy metals such as Arsenic (Ar), Cadmium (Cd), Mercury (Hg) and Tin (Sn) was found to be below detection level (ND: LOQ) & acceptable daily intake as recommended by World Health Organization. In similar illustration, polyherbal formulation exhibited essential minerals such as Copper (Cu), Zinc (Zn), and Manganese (Mn) are found in adequate levels (15.58 - 22.59 mg/kg); while Nickel (Ni), Potassium (K), Phosphorous (P), Chloride (Cl), Calcium (Ca) are measured in significant quantity (0.10 - 1.54 mg/kg).

Table 11: ICP-OES Analysis of ADPFH6 Polyherbal Formulation

<table>
<thead>
<tr>
<th>Element</th>
<th>Levels (mg/kg)</th>
<th>Element</th>
<th>Levels (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic (As)</td>
<td>ND LOQ: 0.25*</td>
<td>Nickel (Ni)</td>
<td>1.54 ± 0.01</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>ND LOQ: 0.25*</td>
<td>Phosphorous (P)</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>0.10 ± 0.01</td>
<td>Potassium (K)</td>
<td>1.13 ± 0.01</td>
</tr>
<tr>
<td>Chloride (Cl)</td>
<td>0.18 ± 0.01</td>
<td>Selenium (Se)</td>
<td>ND LOQ:0.25*</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>22.59 ± 0.01</td>
<td>Tin (Sn)</td>
<td>ND LOQ: 0.25*</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>15.58 ± 0.01</td>
<td>Zinc (Zn)</td>
<td>22.44 ± 0.02</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>ND LOQ: 0.25*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ND: Not Detected / LOQ: Limit of Quantification
3.2.3 SEM-EDX Analysis

Fig. 48 represents the scanning electron micrographs of ADPHF6 sample with particle size of 30 µm. SEM images were further studied for elemental composition using EDX spectral analysis Fig. 49. EDX microanalysis (Table 12) indicates the carbon (C) & Oxygen (O) amounted to be in significant levels of 34 - 56 Wt %; similarly Nitrogen (N) & Potassium (K) were measured to be in reasonable amounts of 2 - 5 Wt %. Microelements such as Copper (Cu), Calcium (Ca), Zinc (Zn), Chlorine (Cl), Magnesium (Mg) & Sodium (Na) yielded in trace amounts of 00.12 to 00.69 Wt %. SEM-EDX analysis also confirmed the absence of heavy metals Viz. Arsenic (Ar), Cadmium (Cd), Mercury (Hg) etc., in ADPHF6 formulation and found to be below detection level (ND: LOQ) & acceptable daily intake as recommended by World Health Organization.

Table 12: SEM-EDAX Analysis of ADPHF6 Polyherbal Formulation

<table>
<thead>
<tr>
<th>Element</th>
<th>Wt %</th>
<th>At %</th>
<th>Element</th>
<th>Wt %</th>
<th>At %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (Ca)</td>
<td>00.50 ± 0.01</td>
<td>00.17 ± 0.01</td>
<td>Oxygen (O)</td>
<td>34.58 ± 0.01</td>
<td>29.71 ± 0.01</td>
</tr>
<tr>
<td>Carbon (C)</td>
<td>55.72 ± 0.01</td>
<td>63.76 ± 0.01</td>
<td>Potassium (K)</td>
<td>02.59 ± 0.01</td>
<td>00.91 ± 0.01</td>
</tr>
<tr>
<td>Chlorine (Cl)</td>
<td>00.18 ± 0.01</td>
<td>00.07 ± 0.01</td>
<td>Selenium (SeL)</td>
<td>00.17 ± 0.01</td>
<td>00.03 ± 0.01</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>00.69 ± 0.01</td>
<td>00.15 ± 0.01</td>
<td>Sodium (Na)</td>
<td>00.12 ± 0.01</td>
<td>00.07 ± 0.01</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>00.17 ± 0.01</td>
<td>00.10 ± 0.01</td>
<td>Zinc (Zn)</td>
<td>00.19 ± 0.01</td>
<td>00.04 ± 0.01</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>05.08 ± 0.01</td>
<td>04.99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig 48: SEM Images of ADPHF6 Polyherbal Formulation

Fig 49: EDX microanalysis ADPHF6 Polyherbal Formulation
3.2.4 LIBS analysis

In our present analysis, LIBS spectra of ADPHF6 polyherbal formulation was recorded by CCD detector equipped with 4-grating to validate the trace elements essential for anti-hyperglycaemic activity. LIBS spectrum has been divided into two fragments with the wavelength ranging from 200-400 nm at 0.1 nm resolutions and the other falls in the wavelength range of 400-800 nm at 0.75 nm resolutions. Fig. 50 & 51 indicates the aggregate of fifty shots of single LIBS spectrum (range: 200-800 nm), with the spectra exhibiting peaks corresponding to Iron I & II (Fe), Carbon (C), Magnesium I & II (Mg), Silicon (Si), Aluminium (Al), Calcium I & II (Ca), Sodium (Na), Hydrogen (H), Nitrogen (N), Oxygen (O). From Boltzmann plot, concentration of elements was calculated (Table 13).

Table 13: LIBS Analysis of ADPHF6 Polyherbal Formulation

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (%)</th>
<th>Element</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium (Al)</td>
<td>3.1 ± 0.02</td>
<td>Magnesium (Mg I)</td>
<td>0.17407 ± 0.06</td>
</tr>
<tr>
<td>Calcium (Ca I)</td>
<td>341.2 ± 0.01</td>
<td>Magnesium (Mg II)</td>
<td>3 ± 0.06</td>
</tr>
<tr>
<td>Calcium (Ca II)</td>
<td>0.77287 ± 0.01</td>
<td>Nitrogen (N)</td>
<td>6 ± 0.06</td>
</tr>
<tr>
<td>Carbon (C)</td>
<td>5.4 ± 0.01</td>
<td>Oxygen (O)</td>
<td>18.9 ± 0.05</td>
</tr>
<tr>
<td>Hydrogen (H)</td>
<td>0.00129 ± 0.02</td>
<td>Potassium (K)</td>
<td>3.1 ± 0.01</td>
</tr>
<tr>
<td>Iron (Fe I)</td>
<td>1.1 ± 0.01</td>
<td>Silicon (Si)</td>
<td>0.0127 ± 0.01</td>
</tr>
<tr>
<td>Iron (Fe II)</td>
<td>616.8 ± 0.1</td>
<td>Sodium (Na)</td>
<td>0.56435 ± 0.01</td>
</tr>
</tbody>
</table>
Fig 50: LIBS Spectra of ADPHF6 Polyherbal formulation ranging 200-400nm

Fig 51: LIBS Spectra of ADPHF6 Polyherbal formulation ranging from 400-800nm
3.2.5 TGA Analysis

Thermal stability and total weight change in ADPHF6 polyherbal formulation was analysed by Thermogravimetric analysis (TGA). Thermogram of the polyherbal formulation in Fig. 52 illustrates the overall decomposition of ADPHF6 powder in different thermal conditions. Initially, ADPHF6 was showed minimal weight loss with 4.32% up to 120°C. Similarly, test material exhibited gradual weight loss of 14.74% up to 240°C. From 240°C to 600°C, polyherbal formulation has shown substantial weight loss of about 53.85%. Decomposition rate of ADPHF6 sample was reduced to 9.551% and remained stable from 600°C to 910°C.

Fig 52: TGA Analysis of ADPHF6 Polyherbal Formulation
3.3 Discussion

Herbal medicine including Indian System of Traditional Medicine, Chinese medicine & other folk medicine gained its reputation globally in recent times for the betterment of human health and also to combat the various fatal disorders (21). Among the western countries, practise of herbal medicine in clinical therapy has been steadily increasing in last two decades since it renders potential pharmacological activity with minimal side effects (22). Though herbal medicine attained the popularity for various ailments, its quality assurance, safety efficacy & drug validation remains a major hindrance for the further progress. As a consequence World Health Organization collaborated with concerned health governing bodies in order to obtain the necessary safety measures of herbal medicine (23). World Health Organization also emphasized the need for safety parameters of herbal medicine & also issued guidelines for the assessment of the quality of herbal medicines through various sophisticated techniques (24). As a measure to standardize our herbal drug, ADPHF6 polyherbal formulation was subjected to GC-MS analysis for identification of active secondary metabolites. To validate our hypothesis, current preliminary investigation of GC-MS analysis also illustrated the presence of 9, 12-Octadecadienoic acid ethyl ester in ADPHF6 polyherbal formulation, which has been reported for playing a vital role in inhibition of carbohydrates hydrolysing enzyme during hyperglycaemic condition.

In recent times there is an increased surge in studying the role of trace elements or micronutrients with concerned to human health (25). In living system roughly 50 essential elements are found to be in assessable range, out of which nearly 23 elements are involved in crucial physiological activities (26). Trace elements also act as catalyst in numerous enzyme and antioxidants systems by abating the oxidative stress (27). Since microelements, especially in the form of metalloproteins are not being synthesised in body, it is a prerequisite as a nutrient supplement to regulate the physiological functions (28). In our present study,
Trace elemental composition was analyzed by ICP-OES & SEM-EDX are found to be comparable and similar in terms of quantitative estimation.

In LIBS analysis, elemental detection by calibration-free (CF) method offers more sensitivity and versatility. Moreover, in LIBS technique all kind of materials are subjected for elemental detection without sample pre-treatment (29). From current analysis, it is evident that Calcium (Ca I) ions, Iron (Fe II) ions, Potassium (K) Copper (Cu), Zinc (Zn), Manganese (Mn), Nickel (Ni) are found to be in adequate levels in ADPHF6. Elements such as Ca, Cu, Mn, and Ni have been reported for its decisive role in diabetes management in various scenarios. Alterations in Ca level have been reported to cause interference in β-cell secretory function & insulin release from β cells of the islets of Langerhans (30). Clinical results suggest, Mn is essential for secretion of insulin and its deficiency leads to Type 2 DM (31). Recent findings validated that K facilitates in conversion of glucose to glycogen, which in turn is stored up in liver during the glycogen and glucose metabolism (32). It has been reported that Cu involves in actively in stimulating the binding of insulin molecule during carbohydrate metabolism and also its deficiency ensued in impaired glucose tolerance (33). Alterations in levels of Zn have been well noticed in insulin release & resistance (34). From the above discussion, it is evident that, these microelements are prerequisite in management of diabetes & its mediated clinical manifestations. Though herbal raw materials manifests numerous significant biological activities, it is believed that such medicinal properties are believed to be dependent of thermal and rheological activity. TGA analysis of lyophilized ADPHF6 powder exhibited minimal weight loss till 240°C and significant thermal decomposition has been observed between 240°C to 600°C.

Trace elements are profoundly involved in carbohydrate metabolism and are essential in the management of diabetes mellitus. To support this hypothesis, our ADPHF6 sample apparently comprise of essential trace elements which accounts for anti-hyperglycaemic
activity. The present study also substantiates, that LIBS can be employed in trace elemental detection from various plant sources. Further studies for optimising the concentration of anti-hyperglycaemic elements using animal models will illustrate the anti-hyperglycaemic activity of polyherbal formulation (ADPHF6).
3.4 References


