

# Application of LIBS in Detection of Antihyperglycemic Trace Elements in *Momordica charantia*

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**Abstract** The present study exploits the information based on concentration of trace elements and minerals in understanding the role/mechanism of action of freeze-dried fruit powder suspended in distilled water of *Momordica charantia* (family: Cucurbitaceae) in diabetes treatment. Laser-induced break down spectroscopy (LIBS) spectra of plant product was recorded under optimized experimental conditions and analyzed. Several atomic lines such as Na, K, Mg, Ca, Fe, Al, etc. have been observed in the LIBS spectra of the above plant product. The concentrations of these minerals are determined by using calibration-free LIBS method. Correlation between the concentration of these elements/minerals and their defined role in diabetes management was studied in normal as well as diabetic animal models.

**Keywords** *Momordica charantia* · Diabetes · LIBS · Indian herbs · Cucurbitaceae

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## Introduction

More than 25% of the pharmaceuticals in use today are derived from natural products. It is, thus, that interest in natural products research that remains strong. Enormous opportunities exist from multidisciplinary research that join forces of pharmacognosy and natural product chemistry, molecular and cellular biology, medicinal and analytical chemistry, biochemistry, pharmacology, and pharmaceutics to exploit the vast diversity of chemical structures and biological activity of natural products<sup>1</sup>. There are significant advantages and opportunities associated with assessing plants as a source of new drugs. There could be a few disadvantages too, but fortunately, the chemical and biological diversity of plants derives the balance in favor of advantages and opportunities and mandates the challenge that disadvantages be addressed and overcome. One of the principle advantages of plants as a source of new pharmaceutical is that the secondary metabolites of plants appear to have evolved over centuries to retain biochemical features that show biological activity<sup>2</sup>. Hence, these plants could also provide a clue for the development of new and better oral drug candidates to tackle diabetes mellitus. One of them is *Momordica charantia* Linn. var. *abbreviata* Ser. (family: Cucurbitaceae), which is commonly known as bitter gourd (melon) or kerala. *M. charantia* is widely planted in tropical areas and is usually consumed as a vegetable. Bitter gourd has also been frequently used as a medicinal herb in Asia, Africa, and South America because of its antidiabetic, anthelmintic, abortifacient, antibacterial, antiviral, and chemopreventive functions<sup>3,4</sup>. Since the activity of plants is related to its constituents (major as well as minor) and their concentrations, a sensitive tool, named as laser-induced breakdown spectroscopy, has been applied to perform the complete elemental analysis of *M. charantia*.

Laser-induced breakdown spectroscopy (LIBS) is a laser-based analytical technique in which qualitative and quantitative analysis of traces present in any material is performed by recording the spectrum of plasma plume generated by focusing a laser beam at the sample surface. It is a versatile, sensitive, real-time, and in situ elemental analysis technique having microanalyses capability for any kind of materials without necessity of sample pre-treatment. LIBS-based elemental analysis of plants will provide a sensitive and efficient tool to analyze the elements present in the plants source. LIBS technique has already been applied for the determination of elemental concentrations in a wide range of materials in the solid, liquid, and gaseous phases<sup>5–10</sup>. It has also been successfully utilized in characterization of material of bio interest<sup>11</sup>. The quantitative analysis using LIBS can be performed, when the calibration curve is available for that particular element embedded in a similar matrix. Thus, for the calibration curve, one should have standard samples having similar matrix as that of sample under investigation, which is difficult to obtain in the case of plant samples. This is the main limitation of LIBS technique for the direct spectrochemical analysis of plant materials. In such a situation, to increase the applicability of LIBS technique in the field of online real-time analysis, calibration-free (CF) LIBS approach<sup>12</sup> may be utilized.

In the present paper, the detection of major and minor elements present in *M. charantia*, a known hypoglycemic herb, have been assessed by using CF-LIBS in terms of its impact on STZ-induced diabetic rats in order to validate the in vivo effect of elements present in *M. charantia* fruits.

## Materials and Methods

### Plant Material

The fruits of *M. charantia* were purchased from the local market of Allahabad, India and authenticated by Dr Satya Prakash, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been submitted. The fruits were freeze-dried at  $-40\text{ }^{\circ}\text{C}$  to get a powder, which was then dissolved in distilled water for experimental work.

### Experimental Animals

Experiments were performed on 6–8-week-old, healthy, albino Wistar rats, of body weight 150–200 g. Animals obtained from National Institute of Communicable Diseases (NICD) New Delhi, India, were housed under standard environmental conditions ( $25\pm 2\text{ }^{\circ}\text{C}$  temperature,  $50\pm 5\%$  humidity with 12 h each of dark and light cycle) and

maintained with free access of water and a standard laboratory diet ad libitum. The study was approved by the Institutional Ethical Committee.

### Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared Streptozotocin (purchased from Sigma Aldrich Chem. Co., USA) 55 mg/kg bw in 0.1 M citrate buffer (pH 4.5) to a group of overnight fasted rats. After 3 days of STZ administration, fasting blood glucose (FBG) and postprandial glucose levels were estimated regularly up to stable hyperglycemia, usually 1 week after STZ injection. Animals having marked hyperglycemia were selected for the study<sup>1</sup>. Blood glucose level (BGL) was estimated by glucose oxidase method<sup>13</sup> using standard kit of Bayer Diagnostics India Limited.

### Assessment of Hypoglycemic Activity in Normal Rats FBG Studies

Four groups of six rats each fasted overnight were used in the experiment. Group I served as control received vehicle (distilled water only). Rats of group II, III, and IV received variable doses of 300, 350, and 400 mg/kg of fruit powder suspended in distilled water, respectively. FBG was taken initially, and then again, blood samples were collected from tail vein at 2, 4, and 6 h after giving the treatment.

### Assessment of Hypoglycemic Activity in Subdiabetic and Mild Diabetic Rats Glucose Tolerance Test Studies

Overnight fasted rats were divided into five groups of six rats each for each diabetic model, sub as well as mild. Group I served as control received vehicle (distilled water only), whereas variable doses of 300, 350, and 400 mg/kg of powder suspended in distilled water were given orally to groups II, III, and IV, respectively, in both the models. Group V serving as a positive control received a dose of 0.5 mg/kg of a known antidiabetic drug, glibenclamide as reference drug. FBG was checked initially, and then BGL was taken after 90 min of treatment considered as “0” h value. A dose of 2 mg/kg glucose was then given orally to all the groups. BGL was further checked up to 3 h at regular intervals of 1 h each, considered as 1-, 2-, and 3-h values.

### Statistical Analysis

Data were statistically evaluated using one-way ANOVA, followed by a post hoc Scheffe’s test using the SPSS computer software, version 7.5. The values were considered significant when  $P < 0.05$ .



**Table 1** CF-LIBS based concentration of elements present in *M. charantia*

Elements	K	Mg	Na	Fe	Ca	Al
Concentration (%) by LIBS	8.42	0.54	2.7	0.22	0.46	0.29
Concentration (%) by AAS	8	0.49	2.4	–	0.39	–

factor,  $I_{\lambda}^{ki}$  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, which takes into account the optical efficiency of the collection system. The concentration of a species is evaluated from the intercept of the Boltzmann plot (by using Eq. 1).

This approach requires an extensive data processing, and a data processing algorithm has been developed<sup>14</sup> to quantify the effect of different CF parameters using a code in MATLAB environment. The program calculates the concentrations of elements in the plasma.

#### Estimation of Elemental Composition

According to general convention of plant material, concentrations of their main matrix elements (Na, K, Mg, Ca, etc.), minor elements (Fe, Al etc.), trace elements, and ultra-trace elements are more than 0.1%, lower than 0.1%, mg/kg, and  $\mu\text{g/kg}$ , respectively<sup>15,16</sup>. All major and minor elements like H, O, N, C, Na, K, Mg, Ca, Fe, and Al are identified, and the concentrations of these elements present in *M. charantia* are evaluated by using CF-LIBS technique, while some of them which have their glycemic potential in diabetic management are tabulated in Table 1. The present results were compared with the results obtained from atomic absorption spectroscopy (AAS). Table 1 clearly demonstrates that the concentrations of trace elements determined with LIBS technique are in close agreement with the values obtained by AAS. Thus, the present result clearly shows that the CF-LIBS technique is suitable for quantitative elemental analysis of plant material. By using the relative concentration of elements (shown in Table 1)

present in *M. charantia*, the quantity (in mg) of different elements responsible for antidiabetic activities in the dose 350 mg/kg of *M. charantia*, which is found the most effective dose, i.e., the dose showing maximum fall in BGL in normal as well as in subdiabetic and mild diabetic models, is evaluated and tabulated in Table 2. Similarly, the quantity (in mg) of different elements responsible for antidiabetic activities in other doses like 300 and 400 mg/kg of *M. charantia* are also evaluated and shown in Table 2. Our result and discussion clearly show that the specific concentration of these elements plays an important role in diabetic management. Function of most of these elements has already been reported in different literature. Most essential trace mineral elements act preliminarily as catalysts or as cofactors in enzyme systems<sup>17</sup>. The role of some inorganic elements like Ca, K, Mg, Al, traces of Fe, etc. in the improvement of impaired glucose tolerance and their indirect role in the management of diabetes mellitus are being increasingly recognized. According to recent reports, specific concentration of K takes part in carbohydrate metabolism; it is active in glycogen and glucose metabolism, converting glucose to glycogen that can be stored in the liver for energy<sup>18</sup>. Mg helps in maintaining this specific amount of K in the cell but the Na and K balance is finely tuned. Mg is a cofactor in various enzyme pathways involved in glucose oxidation. Hypoglycemia is common in patients with diabetes due to excess of urinary Mg losses. Hence, low levels of Mg are commonly seen in the people with diabetes<sup>19</sup>. There is evidence that Mg supplementation may be helpful in insulin resistance<sup>20</sup>. Since its role in glycemic control was unknown, the present study is a forwarding step in this direction. Ca and other traces play an important role in the release of insulin from  $\beta$  cells of islets of langerhans<sup>21</sup>.

**Table 2** CF-LIBS based concentration of elements in different doses of *M. charantia*

Elements	In 400mg/kg dose concentrations of elements (mg)	In 350mg/kg dose concentrations of elements (mg) [effective dose]	In 300mg/kg dose concentrations of elements (mg)
K	6.736	5.894	5.052
Mg	0.432	0.378	0.324
Na	2.160	1.890	1.620
Fe	0.176	0.154	0.132
Ca	0.368	0.322	0.268
Al	0.232	0.203	0.174

## Conclusion

Our results clearly demonstrate that CF-LIBS technique can be used as a basic yet powerful tool for identification of trace elements present in *M. charantia*. It can play a big role in screening of trace elements of various antidiabetic plants for detection of their hypoglycemic elements.

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