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ORIGINAL ARTICLE

The Glycemic Elemental Profile of *Trichosanthes dioica*: A LIBS-Based Study

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Abstract The scientific evaluation of the antidiabetic efficacy of aqueous extract of *Trichosanthes dioica* fruits on streptozotocin-induced diabetic rats is being presented. The graded doses of the extract, viz., 500, 750, 1,000, and 1,250 mg/kg body weight (bw), were administered orally, and it was observed that the blood glucose concentration decreased in a dose-dependent manner. The dose of 1,000 mg/kg bw showed the maximum fall of 23.8% and 19.1% in blood glucose level (BGL) during fasting BGL and glucose tolerance test (GTT) studies, respectively, of nondiabetic rats. Whereas in the case of subdiabetic and mild diabetic models, the same dose showed reduction in BGL of 22.0% and 31.4% during GTT. The study also involves the first use of laser-induced breakdown spectroscopy as a sensitive analytical tool to detect the elemental profile responsible for the antidiabetic activity of aqueous extract of *T. dioica* fruits that exhibits the antidiabetic activity. High intensities of Ca, Mg, and Fe indicate large concentrations of these elements in the extract, since according to Boltzmann's distribution law, intensities are directly proportional to concentrations. The higher concentrations of these glycemic elements, viz. Ca, Mg, and Fe, are responsible for the antidiabetic potential of *T. dioica* as well as other plant already reported by our research group.

Keywords Diabetes mellitus · Glycemic elements · LIBS · Streptozotocin · Albino Wistar rats · *Trichosanthes dioica*

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Introduction

Diabetes mellitus, characterized by hyperglycemia, is the most common metabolic disorder considered among five leading causes of death in the world.[1,2] It is a serious illness with multiple complications and premature mortality, accounting for at least 10% of total health care expenditure in many countries.[3] The long-term hyperglycemic conditions lead to numerous alterations in cell membrane properties; examples are the enhanced rigidity, permeability to cations, and the absolute magnitude of the transmembrane potential.[4] Because the synthetic drugs have undesirable side effects or contraindications, the World Health Organization has recommended the evaluation of traditional plant treatments for diabetes.[5] Hypoglycemic sulfonylureas such as glibenclamide can increase pancreatic insulin secretion from the existing β-cells in streptozotocin (STZ)-induced diabetes by membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion.[6] Moreover, natural plant drugs are considered less toxic and exhibit the side effects less pronounced than those of synthetic drugs.[7–9] Hypoglycemic effects have been reported in some plants that contain terpenoids, iridoid glycosides, flavonoids, and other phenolic compounds.[10]

Trichosanthes dioica Roxb. (family: Cucurbitaceae), commonly known as "Sespadula" in English and "Parwal" in Hindi, is widely grown throughout India.[11] Fruits of this plant are used as vegetable in Indian traditional food system from time immemorial. Besides fruits, other parts of the plant, such as the leaves and tender shoots, have also been used in the traditional system of medicine since ancient times.[12–14] Some specific medicinal properties have been identified, viz., hypocholesterolemic, hypoglyceridimic, and hypophospholipemic when shade-dried fruits were mixed in the food of nondiabetic animals.[12,15] Most recently, its seeds and leaves have also been found as antidiabetic agents by our research group.[16,17] It also serves as a rich source of vitamin C.[12]

The present study is designed to validate scientifically the use of glycemic elements of *T. dioica* in folklore medicines for managing diabetes by evaluating its glycemic potential. The impact was observed on blood glucose level (BGL) of nondiabetic and STZ-induced subdiabetic and mild diabetic rats. The parameters such as fasting blood glucose level (FBG) and glucose tolerance test (GTT) were taken in consideration as valuable scientific tools validating the ethnopharmacological profile of *T. dioica* fruits. Moreover, the present study describes the laser-induced breakdown spectroscopy (LIBS)-based detection of major and minor elements present in *T. dioica* (pointed gourd) fruits responsible for its antidiabetic potential based on screening of the best set of glycemic elements involved.

Experimental

Material Preparation

Fresh unripe fruits (6 kg) of *T. dioica* were purchased from the local market of Allahabad (India) and authenticated by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, India. A voucher specimen (AA512) has been submitted. The fruits were cut into small pieces and shade-dried. The dried pieces were mechanically crushed and extracted with distilled water using soxhlet at boiling temperature (100 °C) up to 36 h. The extract was filtered and concentrated in the rotatory evaporator at 35±5 °C under reduced pressure to obtain semisolid material, which was then lyophilized to get a powder (yield 14.9% *w*/*w*).

Experimental Animals and Induction of Diabetes

Experiments were performed on 6–8-week-old, healthy, male albino Wistar rats, the body weight of which ranged from 150 to 200 g. Animals obtained from the National Institute of Communicable Diseases, New Delhi, India, were housed under standard environmental conditions (25± 2 °C temperature, 50±5% humidity with a 12 h of dark and light cycle each) and maintained with free access of water and a standard laboratory diet (carbohydrates 30%, proteins 22%, lipids 12%, and vitamins 3%) ad libitum. The study was approved by the Institutional Ethical Committee.

Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (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, FBG levels were estimated and postprandial blood glucose level (PPG) checked regularly up to stable hyperglycemia, i.e., 1 week after STZ injection. Animals having marked hyperglycemia were selected for the study[17] and depending upon their BGL divided into two groups, i.e.:

1. Subdiabetic rats with FBG 80–120 mg/dl and PPG more than 210 mg/dl
2. Mild diabetic rats with FBG 120–250 mg/dl and PPG more than 350 mg/dl

Estimation of Glycemic Potential

Blood glucose level was estimated by glucose oxidase method[18] using standard kit (Bayer Diagnostics India Limited). The percentage variation of glycemia for each group was calculated in relation to initial (0 h) values for FBG studies[19] and in relation to control values for GTT studies.[20]

$$\text{FBG \% variation of glycemia} = \frac{G_0 - G_X}{G_0} \times 100$$

G_0 is the initial value; G_X is the value at 2, 4, and 6 h.

$$\text{GTT \% variation of glycemia} = \frac{G_C - G_y}{G_C} \times 100$$

G_C is the control value; G_y is the value at 1 and 2 h

Experimental Design

Initial screening of the aqueous extract was performed by conducting FBG and GTT studies with a range of variable doses in nondiabetic healthy rats for hypoglycemic activity and in subdiabetic as well as mild diabetic rats for antidiabetic activity.

Five groups of six rats each fasted overnight were used in each experiment of FBG and GTT studies of nondiabetic, subdiabetic, and mild diabetic animals. Group 1 served as untreated control receiving vehicle (distilled water), whereas the animals of groups 2, 3, 4, and 5 received the extract suspended in distilled water at doses of 500, 750, 1,000, and 1,250 mg/kg, respectively. For FBG studies of

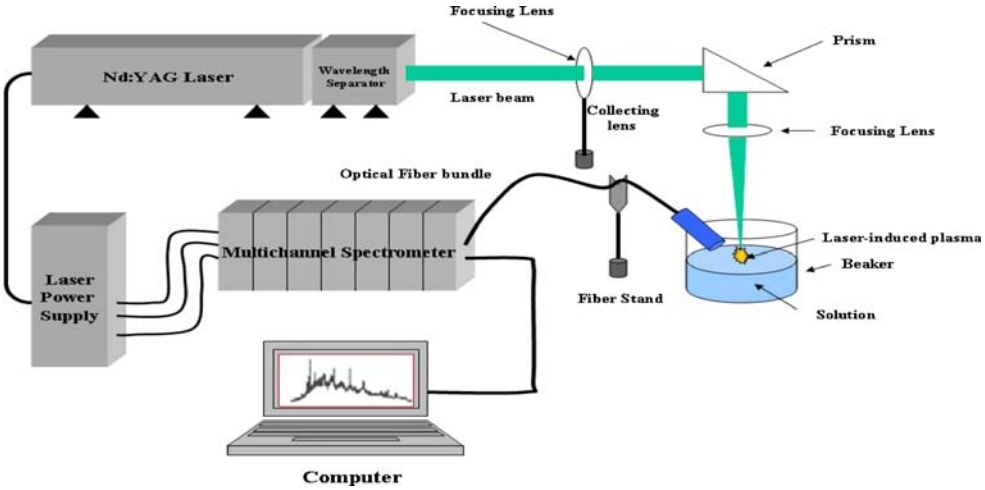

Fig. 1 LIBS experimental setup

nondiabetic and subdiabetic as well as mild diabetic rats, blood samples were collected from tail vain before giving the extract and 2, 4, 6, and 8 h after giving the extract.

For GTT studies on nondiabetic rats, extract was given orally after taking FBG; then, its effect on FBG was studied up to 90 min. The BGL value at 90 min was treated as "0"-h value for GTT. The animals were then orally administrated with 2 g/kg of glucose, and their glucose tolerance was studied at 1-h interval for another 3 h, and the values were considered as 1-, 2-, and 3-h values.

The antidiabetic effect of aqueous extract of *T. dioica* fruits in subdiabetic and mild diabetic rats was also assessed by improvement in glucose tolerance in the same way as above. In this study, one additional group (group 6) was taken as a positive control, and the results were compared with this group of rats, treated with 250 mg/kg of tolbutamide (hypoglycemic agent).

Detection of Trace Elements

A schematic diagram of the experimental setup for recording of LIBS spectra is shown in Fig. 1. The LIBS spectra of *T. dioica* fruit extract powder dissolved in distilled water were recorded to identify the presence of the best set of elements responsible for its antidiabetic efficacy. The four-channel spectrometer equipped with charge-coupled device (CCD; Ocean optics LIBS 2000+) comprising the four gratings was used to get the dispersed light from the plasma. A pulsed laser beam from a Q-switched Nd:YAG laser (Continuum Surellite III-10) was focused on the sample using a converging lens (Quartz) of 30-cm focal length; the temperature of the locally heated region rose rapidly and resulted in plasma formation on the sample's surface. The light emitted from microplasma was collected using an optical fiber tip placed in the vertical

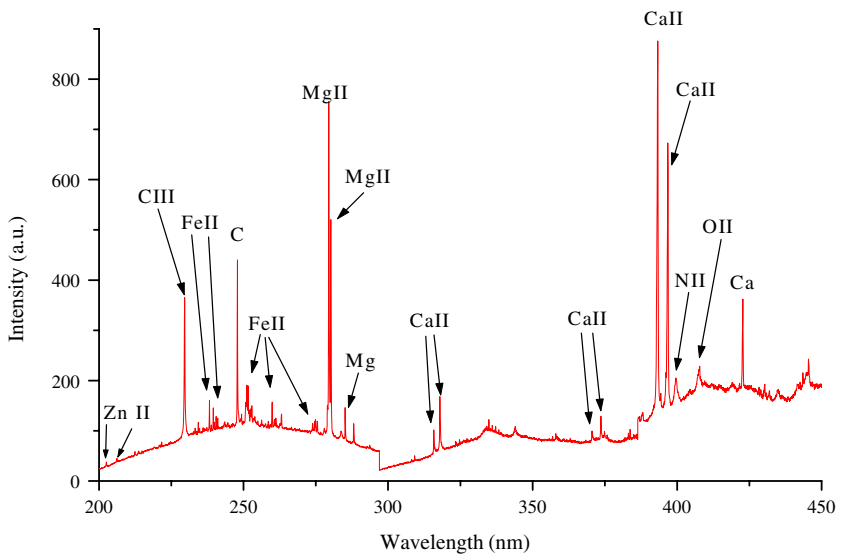

Fig. 2 Laser-induced breakdown spectra of *T. dioica* in the spectral range 200–450 nm

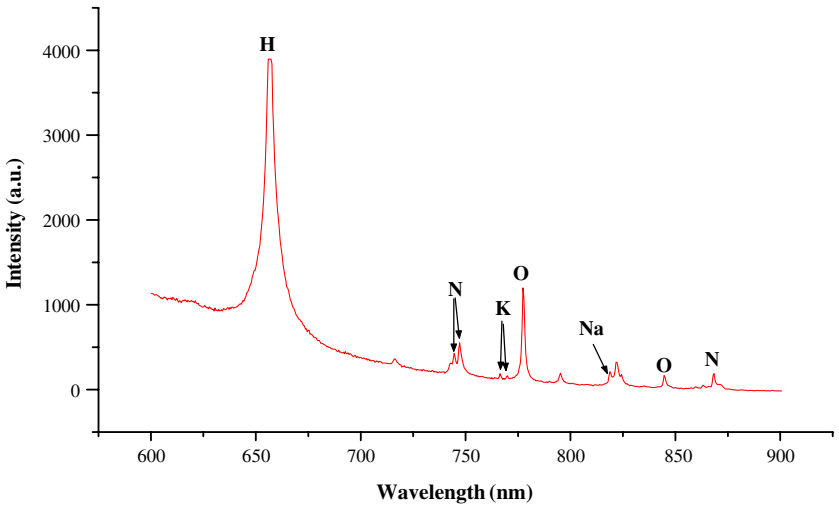

Fig. 3 Laser-induced breakdown spectra of *T. dioica* in the spectral range 200–900 nm

plane (at 45° with respect to the laser beam) and finally fed into the entrance slit of the multichannel spectrometer (Ocean Optics LIBS2000+) equipped with CCD and four gratings. The spectra presented in Figs. 2 and 3 are the averages of 100 scans (100 shots). The first three gratings with 0.1-nm resolution are designed to cover the wavelength range from 200 to 310, 310 to 400, and 400 to 510 nm, respectively, while the fourth grating (resolution 0.75 nm), termed a broadband grating, covered the 200–1,100-nm interval. All four gratings were used simultaneously to record the LIBS spectra. In the case of aqueous extract of *T. dioica*, the LIBS spectra were recorded at the repetition rate of 2 Hz and 175-mJ laser energy. The solution was prepared by dissolving 1.0 g of lyophilized material in 10 ml of distilled water.

Results and Discussion

In the last few years, a number of new oral agents (originating from natural products) for the treatment of type 2 diabetes have been introduced, in the hopes of achieving a better glycemic control. Metformin, a biguanide, is a plant product, which is in clinical use for the treatment of type 2 diabetes for over 40 years. This compound enhances the sensitivity of both hepatic and peripheral tissues to insulin and inhibits gluconeogenesis in the liver.[21] Table 1 describes the hypoglycemic effect of a single oral administration of variable doses (500, 750, 1,000, and 1,250 mg/kg) of aqueous fruit extract in nondiabetic healthy rats. A regular fall and the maximum fall observed in BGL were 23.8% with the dose of 1,000 mg/kg bw.

Table 2 deals with the study of aqueous extract of *T. dioica* fruits on glucose tolerance of nondiabetic healthy rats, and the maximum fall observed at 3 h after glucose administration was 19% when the dose was 1,000 mg/kg.

Table 3 demonstrates the antidiabetic effect of aqueous extract of *T. dioica* fruits on subdiabetic and mild diabetic animals, respectively. Different doses of aqueous extract as mentioned above along with the standard drug tolbutamide 250 mg/kg were given orally to the groups as defined in the experimental design. In the case of subdiabetic rats, the fall of 13.7%, 17.01%, 22.0%, and 16.3% in BGL was observed after 3 h of glucose administration with the doses of 500, 750, 1,000, and 1,250 mg/kg, respectively. The highest fall was observed with the dose of 1,000 mg/kg which is comparable with 24.1% observed with the dose of 250 mg/kg of

Table 1 Effect of graded dose of *T. dioica* fruit aqueous extract on BGL of normoglycemic rats (mean ± SD)

		Blood glucose levels (mg/dl)				
		Pretreatment	Post treatment (h)			
Experimental groups	Treatment (mg/kg bw)	FBG	1.5	3.0	4.5	6.0
Control	Distilled water	69.5±3.9	69.3±3.2	70.0±4.6	70.1±3.8	69.2±4.2
Extract	500	70.0±3.2	68.8±4.4	66.1±4.6	62.4±5.1	60.1±3.8*
Extract	750	68.2±3.2	65.9±4.4	62.1±4.6	58.7±5.1	55.6±3.8*
Extract	1,000	71.4±3.2	68.1±4.4	63.5±4.6	58.9±5.1	54.3±3.8*
Extract	1,250	72.1±4.6	70.4±4.2	67.1±4.8	65.8±3.7*	60.4±4.5*

*$P<0.05$ as compared with initial

Table 2 Effect of graded dose of *T. dioica* fruit aqueous extract on BGL during GTT of normoglycemic rats (mean ± SD)

		Blood glucose levels (mg/dl)					
		Pretreatment	Post treatment (h)				
Experimental groups	Treatment (mg/kg bw)	FBG	1	2	3	4	5
Control	Distilled water	74.3±4.7	73.9±3.6	74.5±4.1	108.3±4.6	98.6±3.2	92.4±4.3
Treated 1	500	68.1±42	67.3±5.1	66.1±4.6	92.4±4.9	84.1±4.4*	78.4±3.8
Treated 2	750	69.1±3.5	68.8±4.9	68.2±3.6	90.8±4.9	82.5±4.6*	76.1±3.7
Treated 3	1,000	70.1±4.2	68.4±4.3	65.8±5.6	90.1±4.9	80.3±4.8*	74.6±4.4
Treated 4	1,250	68.9±4.6	68.0±3.2	67.2±3.8	92.5±4.5*	83.4±3.8*	78.1±3.9

*$P<0.05$ as compared with control

tolbutamide. In the case of mild diabetic animals, the fall observed was 16.4%, 29.7%, 31.3%, and 28.6% during GTT with the doses of 500, 750, 1,000, and 1,250 mg/kg, respectively. However, the doses of 250 mg/kg of tolbutamide reduced BGL by 31.5% which is practically the same as obtained with the dose of 1,000 mg/kg.

The spectra of *T. dioica* fruit extract, shown in Figs. 2 and 3, were taken at optimized experimental conditions. It clearly revealed the presence of Mg, Fe, Na, K, Zn, Ca, H, O, C, and N elements in the spectral range from 200 to 900 nm. According to the Boltzmann's distribution law, intensity is directly related to concentration.[22]

$$\ln \frac{I_{\lambda}^{ki}}{A_{ki} g_k} = -\frac{E_k}{k_B T} + \ln \frac{C_s F}{U_s(T)} \quad (1)$$

where k_B is the Boltzmann constant; λ is the wavelength of the transition; A_{ki} is the transition probability; g_k is degeneracy factor; I_{λ}^{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).

Therefore, the intensity of the observed spectral lines corresponding to major and minor elements present in the extract is not only indicative for their concentration, but it also assists in defining their role in diabetes-induced oxidative stress management. The ratio of intensities of the detected elements (Mg, Fe, Na, K, Zn, Ca, C, H, O, and N) to the intensities of reference lines (C and O that are essential elements of plant materials) was estimated to evaluate their concentration in proportion. Since gratings with different resolutions were used, the whole spectrum was divided in two sections: the first (resolution 0.1 nm) covered the wavelength range from 200 to 510 nm and the second

Table 3 Effect of variable doses of *T. dioica* on GTT of sub diabetic and mild diabetic rats (mean ± SD)

Groups (treatment and doses)	FBG	0h	1h	2h	3h
BGL of subdiabetic animals (mg/dl)					
I (control, D W)	85.5±4.3	84.8±4.7	259.8±3.9	190.2±4.2	131.5±3.6
II (extract, 500 mg/kg)	84.3±5.1	74.6±4.4*	241.0±4.5*	166.5±4.2*	113.4±4.5*
III (extract, 750 mg/kg)	95.9±4.2	94.5±4.7	233.1±4.3*	161.7±4.4**	109.2±4.6***
IV (extract, 1,000 mg/kg)	91.5±4.3	85.6±5.0	203.5±4.8*	149.0±5.1**	102.5±4.8**
IV (extract, 1,250 mg/kg)	93.1±4.1	90.2±4.5	204.9±4.1	162.8±4.7	110.4±4.2
V (tolbutamide, 250 mg/kg)	85.7±4.5	79.6±4.6	200.4±4.9*	145.3±4.3**	99.8±4.4**
BGL of mild diabetic animals (mg/dl)					
I (control, D W)	178.9±3.9	179.2±5.2	398.4±4.7	322.7±5.4	284.6±5.3
II (extract, 500 mg/kg)	176.3±4.5	171.5±5.6	331.2±4.9*	272.7±4.7*	236.7±4.9*
III (extract, 750 mg/kg)	169.4±4.7	166.5±4.8	316.8±5.1*	256.1±5.4**	199.9±5.2***
IV (extract, 1,000 mg/kg)	165.2±5.2	141.5±5.1	259.1±4.6**	214.7± 4.8**	186.9±5.7*
IV (extract, 1,250 mg/kg)	166.6±5.1	159.3±5.3	258.5±5.1	217.4±4.5*	192.4±4.3**
V (tolbutamide, 250 mg/kg)	171.4±4.9	151.3±4.7	259.4±4.6	112.9±4.3	192.1±4.1

*$P<0.05$; **$P<0.001$; ***$P<0.001$, as compared with control

(0.75-nm resolution) spanning the wavelength range between 510 and 1,100 nm. To find the intensity ratios of spectral lines, the C line at 247.88 nm (as a reference line for the spectral range of λ 200–510 nm) and the O line at 844.10 nm (as the reference line for spectral wavelength range of 500–1,100) nm have been selected. Calculated intensity ratios of Zn/C, Fe/C, Mg/C, Ca/C, K/O, Na/O, H/O, and N/O are given in Tables 4 and 5. A broad variety of structurally distinct molecules stimulate insulin secretion from pancreatic β-cells by different mechanisms of action. Esters of succinic acid, the new potent insulin secretagogues,[23–25] have been proposed as a novel antidiabetic agent for type 2 diabetes. It has been previously shown that succinic acid ester can be taken up and metabolized by pancreatic β cells, leading to increased proinsulin biosynthesis,[26] insulin secretion, and lowered blood glucose.[27,28] The possible mechanism of action of the aforesaid elements could be correlated with the reminiscent effect of the hypoglycemic sulfonylureas that promote insulin secretion by closure of K+-ATP channels, membrane depolarization, and stimulation of Ca2+ influx, an initial key step in insulin secretion,[27,28] also protect pancreatic islets in vivo and in vitro against STZ.[29] Recently, we have found in our lab that Mg and Ca manage the blood glucose levels and also restore the antioxidant activity in type 2 diabetic rats.[30–34]

From the outcome of this study, it can be conclusively stated that the higher concentrations of Ca^{++}, Mg^{++}, and Fe^{++}, as reflected by their intensities, are responsible for glycemic potential of *T. dioica*. Further studies to establish the absolute concentrations of trace elements needed for optimum glycemic activity in diabetic management are in progress. In addition, the results obtained demonstrate clearly that LIBS can be considered as a scientific and reliable monitor for detection of glycemic elements in herbs. As such, it can substantially help in the management of diabetes mellitus by screening of various identified antidiabetic medicinal plants for their glycemic elements.

Table 4 Intensity ratio of different elements with respect to carbon (247.8 nm)

Elements	Ratio
Zn (202.5 nm)/C (247.8 nm)	0.02511
Zn (206.2 nm)/C (247.8 nm)	0.01868
C III (229.62 nm)/C (247.8 nm)	1.38261
C (247.8 nm)/C (247.8 nm)	1
Fe II (234.3 nm)/C (247.8 nm)	0.04655
Fe II (238.2 nm)/C (247.8 nm)	0.10883
Fe II (239.5 nm)/C (247.8 nm)	0.08793
Fe II (240.4 nm)/C (247.8 nm)	0.03884
Fe II (249.3 nm)/C (247.8 nm)	0.02752
Fe II (258.5 nm)/C (247.8 nm)	0.0392
Fe II (259.8 nm)/C (247.8 nm)	0.18889
Fe II (260.7 nm)/C (247.8 nm)	0.05208
Fe II (261.1 nm)/C (247.8 nm)	0.29323
Fe II (273.9 nm)/C (247.8 nm)	0.04585
Fe II (274.9 nm)/C (247.8 nm)	0.04928
Fe II (275.5 nm)/C (247.8 nm)	0.05908
Mg II (279.5 nm)/C (247.8 nm)	1.48591
Mg II (280.2 nm)/C (247.8 nm)	1.13031
Ca II (315.8 nm)/C (247.8 nm)	0.10521
Ca II (317.9 nm)/C (247.8 nm)	0.34345
Ca II (393.3 nm)/C (247.8 nm)	3.38716
Ca II (396.8 nm)/C (247.8 nm)	2.02546
Ca II (422.6 nm)/C (247.8 nm)	0.55465
Mg (285.2 nm)/C (247.8 nm)	0.16581
K (766.4 nm)/C (247.8 nm)	0.02045
K (769.9 nm)/C (247.8 nm)	0.01339

Table 5 Intensity ratio of different elements with respect to Oxygen (777.2 nm)

Elements	Ratio
O (777.2 nm)/O (777.2 nm)	1
O (844.6 nm)/O (777.2 nm)	0.07293
H (656.2 nm)/O (777.2 nm)	7.05396
Na (818.3 nm)/O (777.2 nm)	0.0419
Na (589.5 nm)/O (777.2 nm)	0.00796
N (744.2 nm)/O (777.2 nm)	0.05028
N (746.8 nm)/O (777.2 nm)	0.45277
N (868.3 nm)/O (777.2 nm)	0.06613

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References

1. W.H. Gipsen, G.J. Biessels, Trends Neurosci **23**, 542–549 (2000)
2. R. Rahimi, S. Nikfar, B. Larijani, M. Abdollahi, Biomed Pharmacother **59**, 365–373 (2005)
3. H. King, R.E. Aubert, W.H. Herman, Diabetes Care **21**, 1414–1431 (1998)
4. C. Watala, J. Golanski, M.A. Boncler, R. Pietrucha, K. Gwozdzinski, Platelets **9**, 315–327 (1993)
5. C. Day, Br J Nutr **80**, 5–6 (1998)
6. L. Pari, M. Latha, Gen Physiol Biophys **24**, 13–26 (2005)
7. S.M. Sato, Toxicon **39**, 603–613 (2001)
8. A. Aliahmadi, N. Rahmani, M. Abdollahi, Int J Pharmacol **2**, 268–279 (2006)
9. X. Hu, J. Sato, Y. Yu, M. Oshida, G. Bajotto, Y. Sato, Diab Res Clin Pract **59**, 103–111 (2003)
10. W.L. Li, H.C. Zheng, J. Bukuru, N. De Kimpeb, J Ethnopharmacol **92**, 1–21 (2004)

11. Chakravarthy HM (1982) Bot Surv. India pp 136
12. G. Sharma, M.C. Pant, Curr Sci **57**, 1085–1087 (1988)
13. G. Sharma, A. Sarkar, S.B. Pachori, M.C. Pant, Indian Drug **27**, 24–28 (1989)
14. K. Singh, Indian Hortic **34**, 33–37 (1989)
15. S.K. Mukharjee, Indian scenario (Abstract-11), 2–4 September, Varanasi, India (1996)
16. P.K. Rai, D. Jaiswal, S. Diwakar, G. Watal, Pharm Biol **46**, 360–365 (2008)
17. P.K. Rai, D. Jaiswal, R.K. Singh, R. Gupta, G. Watal, Pharm Biol **46**, 894–899 (2008)
18. D. Brahm, P. Trinder, Analyst **97**, 142–45 (1972)
19. L.H. Smith, Med Panam **42**, 1549–1600 (1999)
20. G.S. Kumar, H. Nayak, S.M. Dharmesh, P.V. Salimath, J. Food, Compos Anal **19**, 446–452 (2006)
21. L. Pari, R. Saravanan, IJPT **4**, 132–137 (2005)
22. M. Sabsabi, P. Cielo, Appl Spectrosc **49**, 499–507 (1995)
23. S.F. Picton, P.R. Flatt, N.H. Mcclenghan, Int J Exp Diabetes Res **2**, 19–27 (2001)
24. L.A. Fahiens, M.J. MacDonald, E.H. Kmiotek, R.J. Mertzo, C.M. Fahien, J. Biol, Chemistry **263**, 13610–13614 (1988)
25. M.J. MacDonald, L.A. Fahien, Diabetes **37**, 997–999 (1988)
26. C. Alarcon, B. Wicksteed, Diabetes **51**, 2496 (2002)
27. W.S. Zawalich, K.C. Zawalich, Endocrinology **131**, 649–654 (1992)
28. A. Juan, G. Martinez, L. Maria, V. Penacarrillo, I. Valverde, F. Bjorkling, W.J. Malaisse, Eur J Pharmacol **32**, 565–568 (1998)
29. A.G. Akkan, W.J. Malaisse, Diabet Res **23**, 55–63 (1993)
30. P.K. Rai, N.K. Rai, A.K. Rai, G. Watal, Instrum Sci Tech **35**, 507–522 (2007)
31. P.K. Rai, D. Jaiswal, N.K. Rai, S. Pandhija, A.K. Rai, G. Watal, Lasers Med Sci **24**, 761–768 (2009)
32. Watal G, Sharma B, Rai PK, Jaiswal D, Rai DK, Rai NK, Rai AK (2009) Adv Prot Oxid Str (in press)
33. P.K. Rai, D. Jaiswal, N.K. Rai, S. Pandhija, A.K. Rai, G. Watal, Food Biophys (2009). doi:10.1007/s11483-009-9123-x
34. N.K. Rai, P.K. Rai, D. Jaiswal, S. Pandhija, A.K. Rai, G. Watal, Food Biophys **4**, 167–171 (2009)