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Potential of Aloe vera gel powder against hyperglycemia and hyperlipidemia

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Key words: Anti-hyperglycemic effect, Anti-hyperlipidaemic effect, Atomic absorption spectroscopy, Alloxan induced rats, Laser induced breakdown spectroscopy**.**

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Abstract

Diabetis mellitus is ranked in third position among the fetal diseases in whole world. Now, herbal medication has been considered as safe and less toxic as compared to allopathic medication in treatment of this disease. Aloe vera, due to active polysaccharides, accounts for many biological activities. Anti-hyperglycemic and Anti-hyperlipideamic effect of Aloe vera gel powder were examined by using animal model (rats) in study. Proximate composition, minerals (qualitative and quantitative) of Aloe vera gel powder was examined. Chromium, sodium, iron, calcium, magnesium, manganese, iodine and fluorine were found in Aloe vera gel powder by LIBS and lead, iron, potassium, magenisum, sodium, manganese, and copper were quantified in Aloe vera gel powder by AAS. FBS, TGs, LDL, TC,and HDL were found in normal range after 28 days in group 5 (high dose of Aloe vera gel powder i-e 400mg/kg b.w). Aloe vera gel powder can be used in treatment of Diabetes mellitus.

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Introduction

Diabetic mellitus (DM) is a metabolic disorder due to lack of insulin secretion or insulin action. American Diabetes Association, 2019 published that almost 1.25 million peoples have been suffering from diabetes type 1 only in America and this disease is found in about 44 thousand people in this year. Now it is considered as third fetal disease after cancer and CVDs. Prolonged hyperglycemia may results in various other diseases like kidney failure, weak eye sight, CVDs and in last death (Thiruvenkatasubramaniam and Jayakar, 2010). Proper diet, exercise and medication or insulin therapy helps in management of this disease. Herbal medication is considered less toxic as compared to allopathic medication (Pari and Umamasheswari, 2000). Almost 800 plants have property of anti-diabetic activity due to presence of many bioactive compounds (Ilango and Chitra, 2009).

Aloe vera is the most useful aloe species. In the food industry, *Aloe vera* has been considered as an important functional element in health drinks, functional foods and beverages (Hamman, 2008). The polysaccharides existing in *Aloe vera* extracts are accountable for health benefits (Ni and Tizard, 2004; Ni *et al.,* 2004). *Aloe vera* gel has many biological activities. These are promotion of wound healing, antimicrobial activity, acceleration of radiation damage repair, anti-diabetic effects, antioxidant activity, and stimulation of haematopoiesis, anti-inflammatory, protection of gastrointestinal tract (Talmadge *et al.,* 2004; Reynolds and Dweck, 1999). This study was conducted to determine the proximate composition of Aloe vera gel powder and also its minerals and also to explore the anti-hyperglycemic and anti hyperlipedemic activity of Aloe vera gel powder.

Material and methods

Sample Preparation

Aloe vera leaves (*Aloe barbadensis Miller*) were purchased from local nurseries of Sargodha, Punjab, Pakistan. Selection of leaves was made by considering size, maturity, colour, freshness. Secretion of yellow coloured liquid from the base of cut leaves was drained completely. The outer skin of leaves was then separated from the gel, which was cut by sharp knives into cube pieces of 1 mm in thickness.

Preparation of Aloe vera Gel Powder (AVGP)

Conventional drying was carried out by methods used by Vega *et al.,* 2007 with some modification. Drying of cubes of *Aloe vera* gel was done in convective tray dryer at the temperature 50 ± 5°C with uniform air-flow. The dried samples were then packed in air tight packages for further analysis.

Proximate Analyses of AVGP

AVGP was analyzed for its proximate composition (Moisture, crude fat, crude protein, crude ash, crude fiber, and NFE as explained by standard methods in AOAC, 2005.

Mineral Estimation by Laser Induced Breakdown Spectroscopy

Pallets of sample were made from Industrial Unit, University of Sargodha for LIBS.

Instrumentation of LIBS

The plasma was produced by beam operating at 355nm, having 5-ns pulse duration and 10 Hz repetition rate from Q-switched Nd: YAG laser (Quantal, France). Energy is delivered from this laser upto 230mJ at 355nm, measured by Joule meter (Q-touch, France). The laser beam was targeted on sample, mounting on stage using 25cm focal length quartz lens. To limit the same spot erosion and provide the new surface after every laser shot, the sample on XYZ stage was rotated slowly. The target distance of focusing lens was kept slowly less than focal length of the lens. By using LIBS 2500+ (ocean optics), detection system attached with optical fiber (high OH, Core diameter: 600µm) and collimating lens (0-45 degree view angle), emission from plasma plume was recorded. The placement of collecting lens was normal to direction of expansion of plasma plume for recording the optical emission. The correction of emission signal was made by subtraction of dark signal of detector using OOILIBS software.

There are six spectrometers in LIBS 2500+ system. Each spectrometer has a slit of width 10µm and range from 230 to 805nm. Resolution of spectrometer is approximately 0.1nm. Every spectrometer has also 2048 element linear CCD array.

The synchronization of LIBS 2500+ detection system and Q switch of Nd: YAG laser was made for recording the spectrum emitted from plume. Q switch of the laser and LIBS2500+ via digital delay/ pulse generator were triggered by detection system and flash lamp of Nd: YAG laser respectively in a cycle. The pulse energy of laser and Q switch delay were changed by using OOILIBS software. The calibration of spectrometer was done by using standard light source of DH-2000-CAL. The data acquired by six spectrometer of LIBS 2500+ was stored on computer using instrumental software (Qasim *et al.,* 2016).

Quantification of Minerals by Atomic Absorption Spectroscopy

Minerals in AVGP were quantified by atomic absorption spectroscopy as explained by Rajesakaran *et al.,* 2005 with minor change.

Preparation of ash

100g of moisture free and ground AVGP was placed in crucible and then placed in an muffle furnace set at temperature 440°C. In ashing, all organic materials in sample were ignited then placed in desiccators for cooling.

Analysis of inorganic elements in AVGP ash

Digestion of ash was done in the blend of Hydrochloric acid and nitric acid in 1:3ratio (Rajurkar and Damame, 1998). Then after digestion, the sample was mixed in 50 mL of distilled water and was used for analysis of minerals through atomic absorption spectroscopy.

Anti-Hyperglycemic and anti-hyperlipidemia effect of AVGP (In-Vivo)

Albino rats either sex weighing between 80–110g were used for the biological studies to be conducted in animal house, university of Sargodha, Sargodha. Animals were housed in cages under environmental conditions of 25± 3°C, relative humidity 50± 10% with 12 hours light and dark cycle and had able to get feed and water *ad libitum* freely. Alloxan injection was purchased from local pharmacy and animals will be administered intraperitoneal at dose of 120 mg/kg b.w. to induce diabetes mellitus.

Animals will be randomly divided into six groups having six animals in each group.

Group 1: Positive or normal control without any intervention and received standard feed and distilled water of for 28 days.
Group 2: Negative control group received standard feed, distilled water for 28 days and alloxan injection were administered @ 120 mg/kg body weight (b.w.) intraperitoneal.
Group 3: Animals were received 200 mg/kg b.w AVGP (low dose) once daily by orally for 28 days.
Group 4: Animals were received 300 mg/kg b.w AVGP (medium dose) once daily by orally for 28 days.
Group 5: Animals were received 400 mg/kg b.w. AVGP (high dose) daily by orally for 28 days.
Group 6: Animals were received 50 mg/kg b.w. metformin (standard) once daily by orally for 28 days.

Fasting blood glucose level

All rats were given control, standard and three different doses of AVGP and also kept fasting overnight. Pre-standardized glucometer was used to determine fasting blood glucose levels with reagent strips by glucose oxidase method daily in start and after induction of diabetes, once a week for 4 weeks (Manjunath *et al.,* 2016).

Evaluation of lipids

Blood was collected in tubes containing heparin and then centrifuged to separate the serum 3000 rpm for 5 mins. Serum triglycerides, total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) were evaluated by using Microlab Chemistry Analyzer (300 1x, Merck) with commercially available kits (DiaSys Diagnostic Systems GmbH, Germany) (Yasin *et al.,* 2011).

Statistical analysis

ANOVA, least significance difference and tukey test were used to determine the significant differences ($p \leq 0.05\%$) between different doses of aloe vera gel powder used in current research (Agrahari *et al.*, 2004).

Results and discussion

Proximate Analysis of AVGP

AVGP was exposed for proximate analysis (crude protein, moisture, crude fat, ash, crude fiber and carbohydrates).

Table 1. Proximate Analysis of AVGP.

Sr. No.	Proximate Composition	AVGP (%)
1	Moisture	8.22±0.75
2	Ash	18.65±6.72
3	Crude protein	5.01±1.17
4	Crude fat	3.87±0.393
5	Crude fibre	0.054±0.025
6	Carbohydrates	64.75±6.07

In Table 1, it was seen that AVGP contains moisture 8.22±0.75 %, ash (minerals) 18.65±6.72%, crude protein 5.01±1.17%, crude fat 3.87±0.39%, crude fiber 0.054±0.025%, carbohydrates 64.75±6.07%. Haque *et al.,* 2014 found fat1.83%; protein, 10.50%; ash, 19.50%; carbohydrate, 56.27%. In *Aloe vera* leaves. These results are little bit different from current finding because AVGP was used separately in current study.

Table 2. Different Minerals of AVGP.

Minerals	Concentration of Minerals (mg/g of ash)*
Sodium (Na)	0.0831± 0.0002
Copper (Cu)	0.075± 0.001
Lead (Pb)	0.0723± 0.001
Potassium (K)	0.199± 0.001
Manganese (Mn)	0.319±0.0007
Magnesium (Mg)	0.0783±0.0007
Iron (Fe)	1.74 ± 0.002

*Mean ± standard deviation.

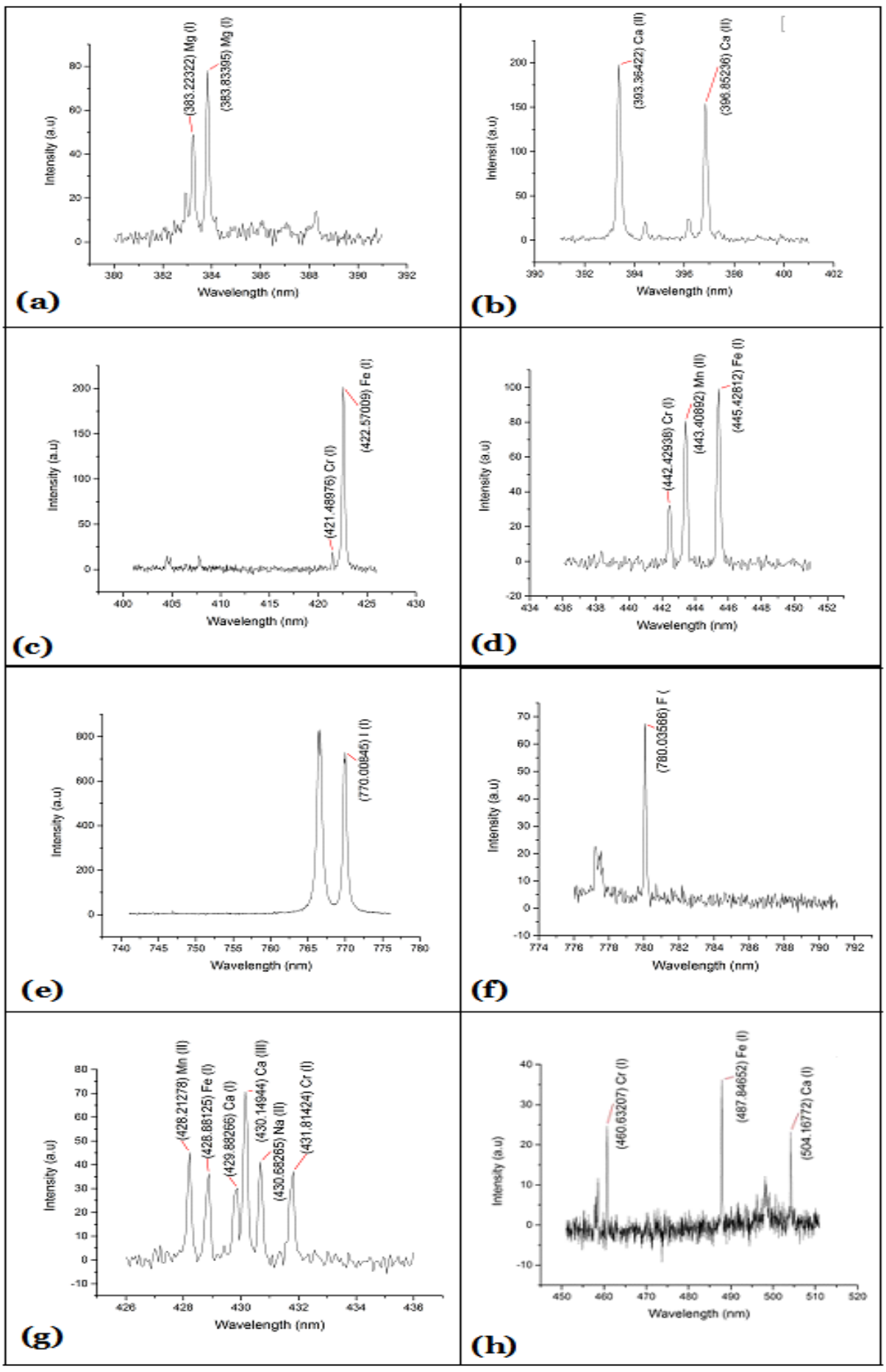
Table 3. Fasting Blood Sugar (FBS), Total Cholesterol (TC), Total Glycerides (TG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) of different groups.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
FBS(mg/dl)	80.67 ± 5.57^{e}	252.00 ± 2.19^{a}	213.83 ± 2.04^{b}	119.83 ± 1.60^{c}	88.50 ± 1.04^{d}	81.67 ± 4.87^{e}
TC(mg/dl)	70.00 ± 3.74^{e}	110.67 ± 7.00^{a}	100.33 ± 3.82^{b}	88.33 ± 2.16^{c}	79.17 ± 2.48^{d}	82.33 ± 5.71^{d}
TG(mg/dl)	70.50 ± 3.77^{d}	94.50 ± 3.50^{a}	88.33 ± 2.16^{b}	79.17 ± 2.48^{c}	74.17 ± 3.18^{d}	70.50 ± 4.76^{d}
HDL-C(mg/dl)	37.83 ± 1.47^{d}	30.17 ± 1.16^{c}	32.33 ± 1.75^{c}	32.833 ± 0.98^{b}	39.83 ± 1.47^{a}	41.00 ± 0.89^{a}
LDL-C (mg/dl)	47.83 ± 3.86^{e}	189.50 ± 11.37^{a}	145.17 ± 6.91^{b}	101.17 ± 6.99^{c}	63.667 ± 4.17^{d}	49.17 ± 6.52^{e}

Small letters show comparison amongst interaction means.

Mineral Analysis of AVGP (Qualitative Analysis)

LIBS technology is the known as rapid and practical technique used to analysis of elements. The main principle of this laser based optical spectroscopy id to identify the atomic and molecular emission signals of elements (Choi *et al.,* 2001). It is simple to analyze the sample at element level even in ppm concentration without any procedure for sampling (Hanafi *et al.,* 2000; Pace *et al.,* 2006).


(383.22322) Mg (I)
(383.83395) Mg (II)
Intensity (a.u)
Wavelength (nm)
(a)
(393.36422) Ca (II)
(396.85296) Ca (II)
Intensit (a.u)
Wavelength (nm)
(b)
(421.48976) Cr (I)
(422.57009) Fe (I)
Intensity (a.u)
Wavelength (nm)
(c)
(442.42938) Cr (I)
(443.40892) Mn (II)
(445.42812) Fe (I)
Intensity (a.u)
Wavelength (nm)
(d)
(770.00845) I (I)
Intensity (a.u)
Wavelength (nm)
(e)
(780.03586) F (
Intensity (a.u)
Wavelength (nm)
(f)
(428.21278) Mn (II)
(428.88125) Fe (I)
(429.88266) Ca (I)
(430.14944) Ca (III)
(430.68265) Na (II)
(431.81424) Cr (I)
Intensity (a.u)
Wavelength (nm)
(g)
(460.63207) Cr (I)
(487.84652) Fe (I)
(504.16772) Ca (I)
Intensity (a.u)
Wavelength (nm)
(h)

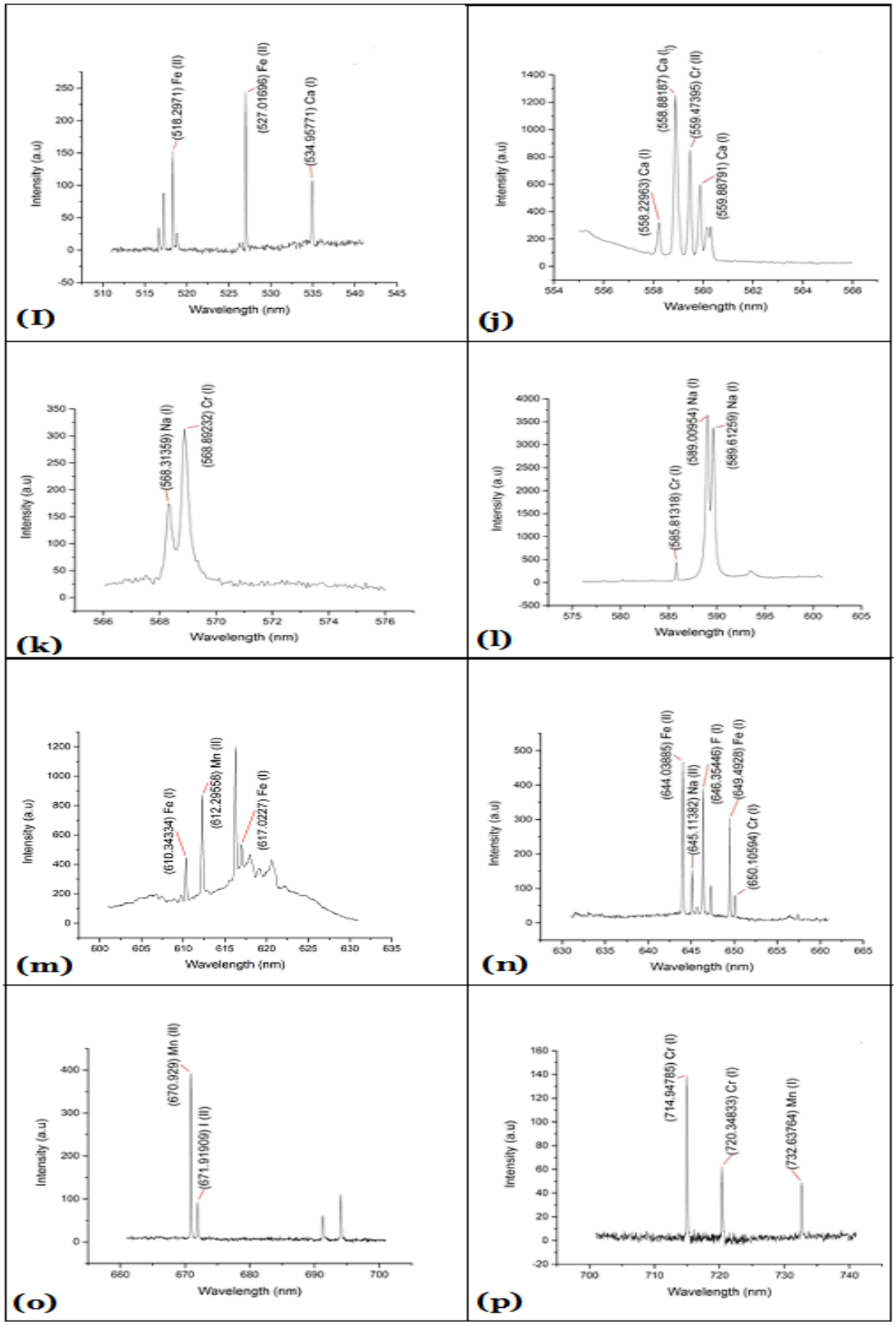


Fig. 1. (a-p left to right): Spectral lines of Aloe vera gel powder by laser induced breakdown of spectroscopy (LIBS) show presence of different minerals.

In LIBS analysis, laser energy is focused on very small volume of sample with a short period of time. This causes the breakdown of sample into atoms which is responsible for characteristic light beam. The emission of light beam was recorded on spectrometer in the form of LIBS spectrum (Bilge *et al.,* 2016).

In food industry, LIBS technology is used to analyze composition of milk powder on element bases (Lei *et al.,* 2011), to detect the presence of contaminants (pesticides) in spinach powder and rice in form of pellets (Kim *et al.,* 2012), to check the presence of some bacteria like *Escherichia coli O157:H7* and *Salmonella enterica* on food surfaces (Multari, *et al.,* 2013), to analyze the sodium in bakery products (Bilge *et al.,* 2015), but some limitations are present in application of LIBS for quantitative analysis.

The spectra obtained were divided in to various ranges as showed in Fig 1(a-p) to identify the lines clearly.

These spectra were analysed by National Institute of Standards and Technology atomic data base (NIST, Atomic spectra database). Calcium (Ca), Iron (Fe), Magnesium (Mg), Iodine (I), Flourine (F), Chromium (Cr), Sodium (Na), Manganese (Mn) were found in AVGP while studying the spectral lines.

Mineral Analysis of AVGP (Quantitative Analysis)

From Table 2, it was observed that AVGP contains iron 1.74 ± 0.002mg /g of ash, copper 0.075± 0.001 mg/g of Ash, Manganese 0.319 ± 0.0007, potassium 0.199±0.001, magnesium 0.156±0.0015, lead 0.0723±0.001 sodium 0.083± 0.0002 mg/g of Ash. Similar results were found by Rajasekaran *et al.,* 2005. Sodium and potassium helps in balancing the electrolite ions and also in kidney diseases (Rajurkar and Damme, 1998).

The function of copper is in insulin binding and its deficiency may be result in increseased blood sugar levels (Mertz, 1993). Chromium plays an important role in carbohydrates and lipids metabolsim and its deficiency results in diabetes mellitus (Mertz, 1993).

Anti-Hyperlipidaemia and Anti-Hyperglycemic Activity of AVGP

Hyperglycemia and hyperlipidaemia are two major consequences of diabetes mellitus; one of the most common chronic disease across the globe. The mechanism behind the induction of diabetes by alloxan is that the β-Cells of pancreas, secreting insulin damage and results in decreasing insulin release, then rats are suffering from hyperglycemia in short time and also over production of hepatic glucose. Besides diabetes mellitus induction, interperitoneal injection of alloxan at dose of 120mg/kg b.w results in hyperglycemia condition, polyphagia, hyperlipidemia, polydipsia, and weight loss as compared to healthy rats (Rajagopal and Sasikala, 2008).

TC, TG, Free Fatty Acid, Phospholipids, LDL, VLDL and AL levels are significantly increased by Alloxan. As insulin inhibits hormone sensitive lipase, free fatty acids mobilization increases in diabetic condition. Lipolytic hormones on the fat were also uninhibited resulting hyperlipidaemia in diabetic condition (al-Shamaony *et al.,* 1994). Alloxan may cause the fatty acids to convert in to phospholipids and cholesterol. These two substances, phospholipids and cholesterol alongwith excess of TG in liver may be released into lipoprotein in blood (Bopanna *et al.,* 1997).

In Table 3, FBS was reduced in group 3, 4, 5, as compared to negative control (group 2) by ingestion of AVGP significantly. But in group 5, the fasting blood sugar was normalized at high dose (400 mg/ kg b.w) of AVGP after 28 days.

Current results were found similar with the results of Manjunath *et al.,* 2016 who found decrease of blood glucose level with Metformin and *Aloe vera* leaf extract after 5 weeks of study. Other studies were also reported that *Aloe vera* gel extract had hypoglycemic effect in streptozotocin induced rats (Rajeskaran *et al.,* 2004; Noor *et al.,* 2008). Another study was also supported the anti-hyperglycemic effect of *Aloe vera* extract in alloxan induced diabetic rabbits (Akinmoladun and Akinloye, 2007).

Hyperlipidaemia is the major consequence of diabetes. This study also showed that TC, TG, and LDL were increased and HDL was decreased by alloxan induced diabetes. A decrease in TC, TG, and LDL and increase in HDL by AVGP were found significantly and HDL was increased and LDL was decreased by AVGP significantly. There were no significantly difference between Group 5 (high dose 400 mg/kg b.w) and Group 6 (metformin 50mg/kg b.w) in TC, TG and HDL levels.

These results were in accordance with Mohamed, 2011 who confirmed the antihypercholestermic effect of *Aloe vera* gel extract in Alloxan induced Diabetic rats.

Rajaskaran *et al.,* 2006 established that *Aloe vera* gel extract not only reduced fasting blood sugar but also reduced the TC, TG, and FFAs in plasma, liver and kidneys of Streptozotocin induced diabetic rats. Processed *Aloe vera* gel decreased in serum TG significantly for 8 weeks (Kim *et al.,* 2009).

Conclusion

It was concluded that Aloe vera gel powder contains almost all micro and macro minerals which play an important role in carbohydrate and lipid metabolism.

Aloe vera gel powder functions as hypoglyceamic and hypolipidaemic agent. It should be recommended that Aloe vera gel powder is used as functional ingredient in many food products.

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