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Study on element content of some Antidiabetic medicinal plants grown in North East India by Atomic Absorption Spectroscopy (AAS) and Flame Photometry

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Abstract : Diabetes is rapidly emerging as serious and major public health-care problem throughout the world. Diabetes mellitus has been found to be managed by various medicinal plant extracts. Many plant derived products are used as antidiabetic supplements. Among the factors attributable to the antidiabetic potential of these medicinal plants, are the various trace elements present in them. The aim of the present study was to investigate the content of trace elements in three antidiabetic medicinal plants traditionally used to manage diabetes mellitus in North East India by Flame Photometry andAtomic Absorption Spectroscopy (AAS) techniques. The elements such as sodium, potassium, iron, manganese, zinc, chromium, copper and nickel were identified and quantified which play vital roles in blood glucose reduction.This study suggested that the analyzed medicinal plants are potential sources for providing a reasonable amount of the required elements to the patients of diabetes mellitus.
Keywords : *Aegle marmelos*,*Musa paradisiaca, Garcinia pedunculata*, Atomic Absorption Spectroscopy (AAS), Flame Photometry, diabetes mellitus, trace elements, medicinal plants.

1. Introduction

Diabetes mellitus is a metabolic disturbance of carbohydrates, proteins and fat due to relative lack or complete absence of insulin and insulin resistance [1]. It is a complex and chronic illness. It is characterized by high levels of blood glucose level. A large number of synthetic oral antidiabetic drugs are used for the treatment of diabetes. However, these drugs have many drawbacks and prominent side-effects or even toxic effects [2]. Plants are found to be one of the most important sources of medicines. Medicinal herbs are major source of many drugs which are used for the treatment of various health problems. There are numerous traditional medicinal plants reported to have antidiabetic property [3]. From available literature it has been found that there are more than 400 medicinal plant species having hypoglycemic activity [4]. Assam is one of the seven states of North East India. Among the seven states of North-Eastern region of India, Assam has distinct and some special features relating to its climate, demography, soil topography etc. Heavy rainfall takes place during monsoon. A large area of the state is covered by dense semi-evergreen to evergreen forests. Assam is one of the richest biodiversity zones in the world.

A large number of plant species having antidiabetic activity are available in various parts of Assam. *Aegle marmelos*is a medium sized subtropical aromatic tree of rutaceae family. This plant is known as Wood apple or Bael tree. It is a valuable medicinal plant. Almost all parts of this plant are used as traditional folk medicine against different human ailments. It is reported that rural community in India are using *Aeglemarmelos* leaf for treating diabetes and related symptoms in herbal folk medicines [5,6]. *Musa paradisiaca* is widely distributed throughout the tropical regions. It is commonly known as banana tree or kela tree. It is found all over the India. It is a tall herbaceous plant. Fruits are found to be fleshy, oblong, 6-8cm long in wild form and more longer in the cultivated varieties. It is reported that mature green fruit of *Musa paradisiaca* are used to treat diabetes in traditional herbal medicine [7,8]. *Garcinia pedunculata* is a large evergreen tree. This tree is most commonly available in North East India [9]. The ripe fruit is golden yellow in colour and 8-12 cm in diameter. The mature ripe fruits are eaten cooked or raw. In Assam it is locally known as Borthekera. *Garcinia pedunculata* has various medicinal properties. It is reported that rural community of peoples in Assam are using dry ripe fruit pulps of this plant for treating diabetes in herbal medicines [10,11].

Many trace elements are found to play an important and vital role in the maintenance of normal blood sugar level. Research studies have revealed that there is a direct association of macro and trace elements with diabetes mellitus [12]. Insulin action on reducing blood glucose level was found to be potentiated by some trace elements [13]. Vanadium, zinc, chromium, copper, iron, potassium, calcium, sodium, manganese and nickel are found to be some of such elements which are reported to take significant part in the control and management of diabetes mellitus [14]. Traditional medicinal herbs are known to have many essential and nutritional elements. Many important mineral elements remain chelated with various organic ligands and thus make them bioavailable to the human body system. The hypoglycemic potential of different herbal remedies may be attributable to the various mineral elements present in them [15].

There may be variation of elemental compositions of the same plant species in different regions. This variation is mainly due to the differences in the characteristics of soil and the different mineral composition of the soil where the plant species are grown. Studies on elemental composition as well as characterization of elements of these plant species collected from this region of Assam has not yet been done properly. As there is no study available in the literature regarding the metal content of many antidiabetic plant species grown in the studied region of Assam, therefore the present study was designed to analyze and characterize the major elements of a few antidiabetic plant species. This study was designed in order to determine the content of antidiabetic trace elements in three medicinal plants traditionally used in management of diabetes mellitus in Assam namely: *Aegle marmelos, Musa paradisiac* and *Garcinia pedunculata.*Flame Photometry and Atomic Absorption Spectroscopy (AAS) techniques have been employed to analyze the levels of elements in these anti-diabetic medicinal plants.

2. Materials and Methods

2.1 Plant materials

Fresh leaves of *Aegle marmelos* were randomly collected from one individual tree. Fresh ripe fruits of *Garcinia pedunculata* and matured green unripe fruit of *Musa paradisica* were also obtained. The parts of interest of these plants were thoroughly washed in deionized water to remove dirt and other contamination due to dust and environmental pollution. After, the leaves were kept for 5-6 hours for the water to dry off. Then the leaves were dried under shade and ground into powder. The fruits were peeled off. The fruits were cut into small pieces. Then the pieces of the fruits were dried under shade. The pieces were mechanically crushed and ground into powder.

2.2 Sampling area

The samples of the plant materials were freshly collected from different local village areas of Nalbari district, Assam, India. Nalbari district is located in between 91°E and 91°47′E longitude and 26°N and 27°N latitude. The plants were identified and authenticated in the Department of Botany, Gauhati University. The parts of interest of these plants were collected during the month of July to October.

2.3 Procedure for sample preparation for analysis in Atomic Absorption Spectroscopy and Flame Photometry

Dried powder of plant material was weighed and taken in ceramic crucible and heated in an electric Muffle furnace at 450 °C for 5 hours. Ashing have destroyed all of the organic materials present in the sample. The crucibles containing ash was then taken out of the muffle furnace and kept in a desiccator. Then the ash was cooled and their weights were taken. Then a mixture of HNO_3, HCl and H_2SO_4 in a 1:2:4 ratio was added in portions to the sample of the ash obtained from the plant part. The mixture was digested over an electric hot plate taking in a porcelain basin for about 2 hours till the black colour of the residue vanished. The pasty colourless residue was dissolved in about 12 ml of 1:1 conc. HCl and H_2O mixture. The resultant mixture was then allowed to stand for overnight and filtered into a 100 ml volumetric flask through whatman 40 filter paper to remove the trace of colourless insoluble solids still present, and the volume was made upto 100 ml mark. The solution so prepared was taken for AAS and FP.

2.4 Atomic Absorption Spectroscopy

The prepared samples of dilute filtrate solutions were used for the analysis of elements of interest by Atomic Absorption Spectroscopy (AAS). Perkin Elmer AAnalyst 200 model was used for Atomic Absorption Spectroscopy. Suitable hollow cathode lamp was used for analysis. The concentration of different elements was analyzed by relative method using A.R. grade solutions of different required elements of interest. The standard conditions for atomic absorption measurement are represented in the following Table1.

Table 1.Standard conditions for Atomic Absorption measurement

Element	Wavelength (nm)	HC Lamp current (mA)	Slit width (nm)	Optimum working range (ppm)	Types of Flame
Fe	248.3	5	0.2	0.06-15.0	Air-C_2H_2
Cu	324.8	4	0.5	0.02-3.0	Air-C_2H_2
Zn	213.9	5	1.0	0.01-2.0	Air-C_2H_2
Ni	232.0	4	0.2	0.1-20.0	Air-C_2H_2
Mn	279.5	5	0.2	0.02-5.0	Air-C_2H_2
Cr	357.9	7	0.2	0.06-15.0	Air-C_2H_2

2.5 Flame Photometry (FP)

Na and K were estimated by using Flame Photometer. Elico Flame Photometer of CL-361 Model was used for Flame Photometry.

3. Results

Table 2.Weight of the crude sample and ash of the *Aegle marmelos*leaves.

Sample	Weight of dried powdered leaves	Weight of ash of the leaves
*Aeglemarmelos*leaves	1.5 g	0.168 g

Table 3.Weight of the crude sample and ash of the unripe fruit pulp of *Musa paradisiaca*.

Sample	Weight of dried powdered unripe fruit pulp	Weight of ash of the unripe fruit pulp
*Musa paradisiaca*unripe fruit pulp	2.5 g	0.105 g

Table 4.Weight of the crude sample and ash of the ripe fruit pulp of *Garcinia pedunculata*.

Sample	Weight of dried powdered ripe fruit pulp	Weight of ash of the ripe fruit pulp
*Garcinia pedunculata*ripe fruit pulp	2.5 g	0.175 g

Table 5.Concentrations of elements by Atomic Absorption Spectroscopy and Flame Photometry in ash sample of*Aegle marmelos*leaves.

Elements	ppm in 100 ml of aqueous extract of the ash
Fe	0.5495
Cu	0.0285
Ni	0.0675
Mn	0.423
Zn	0.185
Cr	0.2195
Na	1.35
K	15.2

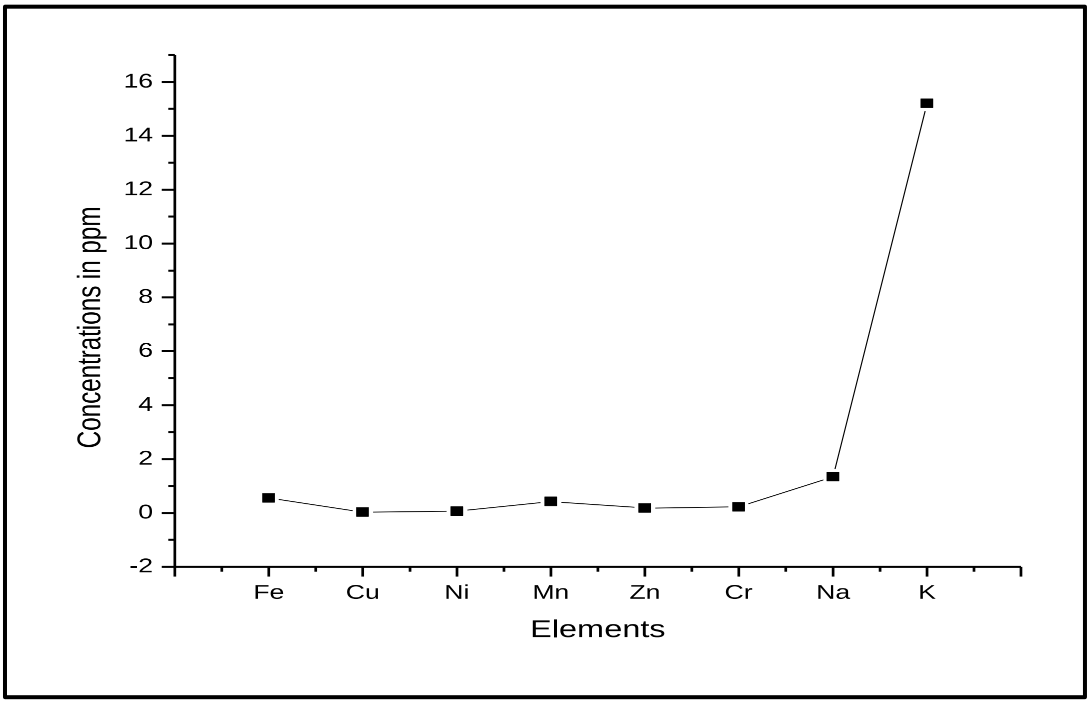

Fig. 1.Concentrations of elements in ash sample of *Aegle marmelos*leaves.

Table 6.Concentrations of elements by Atomic Absorption Spectroscopy and Flame Photometry in ash sample of unripe fruit pulp of *Musa paradisiaca*.

Elements	ppm in 100 ml of aqueous extract of the ash
Fe	1.619
Cu	0.052
Ni	0.039
Mn	0.609
Zn	0.389
Cr	0.376
Na	1.375
K	35.4

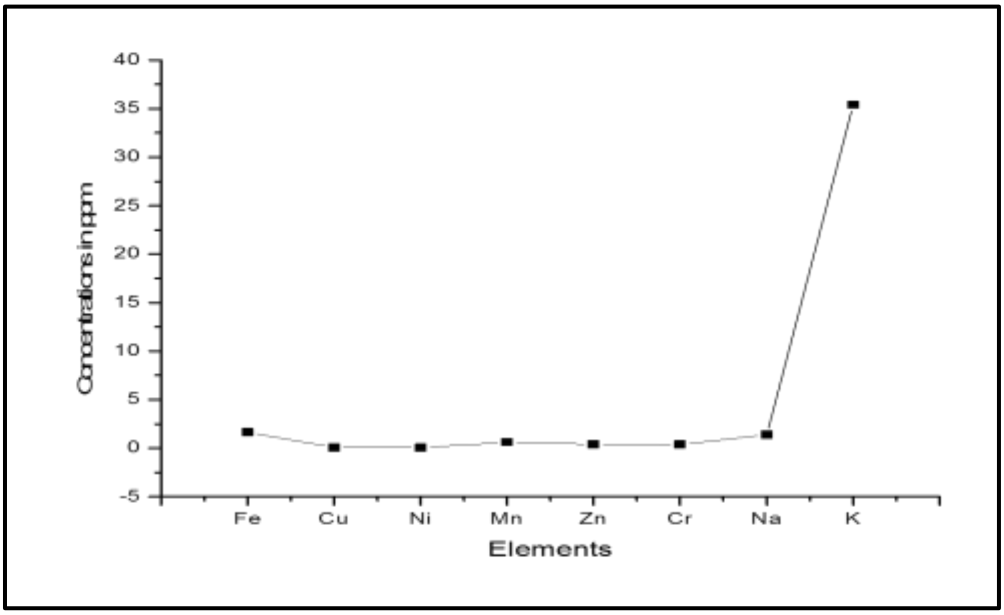

Fig. 2. Concentrations of elements in ash sample ofunripe fruit pulp of *Musa paradisiaca*

Table 7.Concentrations of elements by Atomic Absorption Spectroscopy and Flame Photometry in ash sample of ripe fruit pulp of *Garcinia pedunculata.*

Elements	ppm in 100 ml of aqueous extract of the ash
Fe	0.179
Cu	0.024
Mn	0.019
Zn	0.154
Cr	0.031
Na	0.716
K	16.06

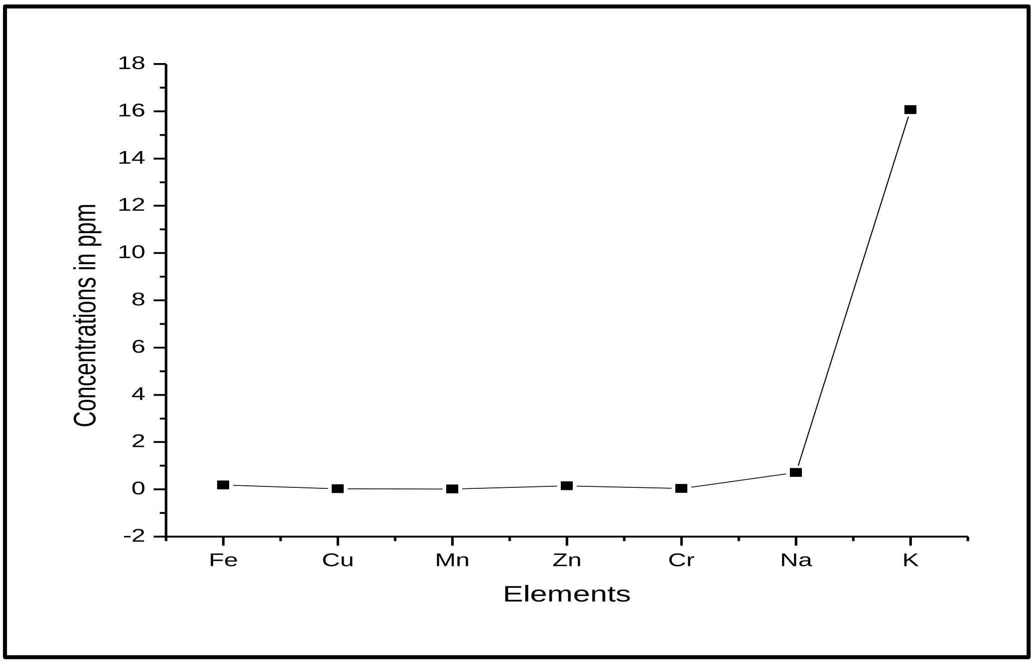

Fig. 3.Concentrations of elements in ash sample of ripe fruit pulp of *Garcinia pedunculata.*

4. Discussion

In the present investigation, analysis of trace elements was done to determine the content of different elements in the selected antidiabetic plants. The concentrations of various elements as analyzed by Atomic Absorption Spectroscopy (AAS) and Flame Photometry (FP) are shown in tables: 5, 6 &7. From the results of this analysis, it was found that most of the investigated elements were present in detectable quantities in the plant species. The concentrations of the elements are presented in parts per million (ppm). The elements K, Na, Fe, Mn, Zn, Cr, Cu and Ni were analyzed by Atomic Absorption Spectroscopy and Flame Photometry technique by measuring the absorbance of the elements at its corresponding resonance wavelength. The concentration of various elements analyzed in the *Aegle marmelos* leaves decreases in the order: K>Na>Fe>Mn>Cr>Zn>Ni>Cu. The concentration of various elements analyzed in unripe fruit pulp of *Musa paradisiaca* decreases in the order: K>Na>Fe>Mn>Zn>Cr>Cu>Ni. In ripe fruit pulp of *Garcinia pedunculata* the decreasing order of concentration of various elements is: K >Na > Fe >Zn >Cr>Cu>Mn. Flame Photometry is one type of atomic spectroscopy where the species examined in the spectrometer are basically in the form of atoms. Flame photometry is useful for qualitative and quantitative analysis of several cations. Cations of group I and Group II metals are generally good candidates for analysis in Flame Photometry because of their low excitation energies. Atomic Absorption Spectrometry (AAS) is another good analytical technique that can measure the concentration of a particular metal element within a sample.

Globally an estimated number of about 387 million people are currently diagnosed to have diabetes [16]. WHO projected that diabetes will be the 7th leading cause of death in 2030 [17]. Currently in India the number of diabetic patients are around 40.9 million and it is expected to rise to 69.9 million by 2025 [18]. Therefore, management of diabetes mellitus is of prime concern in the current scenario. There has been an increasing demand of herbal antidiabetic medicines due to their low cost, easy availability, effectiveness and lesser side effects. Indigenous medicines have been used in the control and treatment of diabetes from the time of Charaka and Sushruta in India [19]. Different parts of medicinal plants are used in the herbal preparations.

From different studies it has been found that supplementation of a single element like calcium, zinc, potassium, manganese or selenium could efficiently alter blood glucose levels of human body. Similarly, combined effect of the mixture of all these elements present in these studied antidiabetic plants should produce a much greater effect than supplementation by a single trace element. Trace elements present in the antidiabetic plants might be in the various organic forms. Thus, elements in different plant parts are more readily bioavailable than through different inorganic forms of supplementation of these elements. The trace elements individually may not produce hypoglycemia in diabetic patients. But their consumption with several metals containing compounds and through different chemical forms present in various herbal products might play a major and vital role.

The proposed mechanism of trace elements of enhancing insulin action includes activation of insulin receptor sites, serving as cofactors for enzyme systems involved in glucose metabolism [20], enhancing insulin sensitivity and acting as antioxidants preventing tissue peroxidation [21]. Some of the trace elements, such as zinc, chromium, copper, manganese, vanadium and selenium are found to play a major role in protecting the insulin secreting pancreatic β-cells which are very much sensitive to free radical damage [22].Sodium (Na) involves in the transport of glucose into the body cells. Potassium supplementation is reported to improved insulin sensitivity, responsiveness and secretion [23]. Recent studies have shown that the low potassium concentration may be a possible risk factor for diabetes [24]. Potassium depletion may result in reduced glucose tolerance [25**]**. In human body K and Cl act as electrolytes. Potassium is essential, mainly in the intercellular fluid as the primary ion. Sodium together with potassium helps to regulate the water balance within the body. It regulates the transfer of nutrients to the cell, transmits electrochemical impulses and is necessary for the normal growth and most of the enzymatic reactions. Recommended daily intake of sodium and potassium for an adult person is 2.4 g and 3.5 g respectively.

Zinc is required for proper glucose metabolism. Zn is used as a cofactor for the function of intracellular enzymes that may be involved in lipid, protein and glucose metabolism [26]. Zinc plays important role in the biosynthesis, storage, utilization and secretion of insulin [27]. Zinc also has a protective effect against β-cell destruction. From research studies, it has been found that zinc maintains the structural integrity of insulin [28]. Zinc also influences the enzyme called glyceraldehyde-3-phosphate dehydrogenase which is involved in glycolysis [29]. Diabetic patients typically excrete excessive amounts of zinc in their urine and therefore supplementation is required [30]. Recommended daily intake of zinc for an adult person is 15 mg. The upper level of zinc intake for an adult man is set at 45 mg/day. In our present study the concentration of zinc in *Aegle marmelos, Musa paradisiaca* and *Garcinia pedunculata* is 0.185 ppm, 0.389 ppm and 0.154 ppm respectively. In view of the positive role of zinc on pancreatic beta cells and insulin, the use of these antidiabetic plant species in the treatment of diabetes mellitus may be attributed to considerable amounts of zinc present in them.

Manganese is essential for glucose metabolism and deficiency of manganese may result in glucose intolerance similar to diabetes mellitus in some animal species [31]. The amount of manganese required by normal adult human being is 5.5 mg per day. In experimental animal models, pancreatectomy and diabetes have been correlated with decreased concentration of manganese in blood [25]. Mn was found in detectable quantities in the leaves of *Aegle marmelos* (0.423 ppm), fruit pulp of unripe *Musa paradisiaca* (0.609 ppm) and ripe fruit pulp of *Garcinia pedunculata*(0.019 ppm).Copper is considered as a powerful enzyme catalyst. Copper possesses an insulin-like activity and promotes lipogenesis[32].Abnormal copper metabolism can lead to several chronic pathogenesis, such as diabetes or diabetic complications [33].The distributions of the concentration of Cu in the studied plants are shown in table. The concentration of Cu ranges from 0.052 ppm to 0.024 ppm.

Iron influences glucose metabolism and insulin action. Iron interferes with insulin inhibition of glucose production by the liver[34].It facilitates insulin binding and subsequent uptake of glucose molecules into the body cell and therefore decreases fasting glucose levels, enhances glucose tolerance and decreases total cholesterol in type II diabetic patients [30].It has been found that chromium administration decreases fasting and postprandial glucose concentration. It can also decrease fatigue, excessive thirst, and frequent urination. The concentration of Fe was 0.5495 ppm for the leaves of *Aegle marmelos*, 1.619 ppm for fruit pulp of unripe *Musa paradisiaca* and 0.179 ppm for ripe fruit pulpof *Garcinia pedunculata*in our present study. The concentration of Cr was 0.2195 ppm for the leaves of *Aegle marmelos*, 0.376 ppm for fruit pulp of unripe *Musa paradisiaca* and 0.031 ppm for ripe fruit pulpof *Garcinia pedunculata*in our present elemental analysis.

Our results have shown that the analyzed antidiabetic plants can be considered as a potential and reliable source of different required elements for patients with diabetes mellitus. The results of the present study of elemental analysis have supported the traditional usage of these plants in the treatment of diabetes. The presence of all these elements (namely Fe, Cr, Cu, Mg, Zn, Na etc.) in the plants studied reveals the importance in diabetes control and justification for inclusion for further study.

5. Conclusion

Medicinal plants contain various trace elements in bioavailable form. In this study, Atomic Absorption Spectroscopy and Flame Photometry were used for quantitative analysis of trace elements in the leaves of *Aegle marmelos*,unripe fruit pulp of *Musa paradisiaca* and ripe fruit pulp of *Garcinia pedunculata*. Concentrations of K, Na, Fe, Mn, Zn, Cr, Cu and Ni were determined in the three antidiabetic medicinal plants grown in North East India. The present analysis has established that the studied antidiabetic medicinal plants have appreciable quantities of some of the important trace elements associated with the blood glucose lowering effects and the regulation of insulin in the human body. The presence of various elements detected in the present investigation has proved the usefulness of these medicinal plants in diabetes treatment.

6. References

1. Sitorus P., Characterization Simplisia and Ethanolic Extract of Pirdot (*Saurauia Vulcani*, Korth) Leaves and Study of Antidiabetic Effect in Alloxan Induced Diabetic Mice, International Journal of PharmTech Research,2015, 8(6): 789-794.
2. Yogalakshmi K., Vaidehi J., Ramakotti P., Hypoglycemic Effect of *Gymnema sylvestre* LeafExtract on Normal and Streptozotocin Induced Diabetic Rats, International Journal of PharmTech Research, 2014, 6(12): 5146-5150.
3. Devi S., Kumar D., Kumar M., Ethnobotanical values of antidiabetic plants ofM.P. region, India, Journal of Medicinal Plants Studies,2016, 4(3):26-28.
4. Patel D.K., Prasad S.K., Kumar R., An overview on antidiabetic medicinal plants having insulin mimic property, Asian Pacific Journal of Tropical Biomedicine, 2012, 2(4): 320-330.

5. Jhajhria A., Kumar K., Tremendous pharmacological values of *Aegle marmelos*,International Journal of Pharmaceutical Sciences Review and Research, 2016, 36(2): 121-127.
6. Patil M.B., Anti-diabetic activity of some medicinal plants, Indian Journal of Applied Research, 2016, 6(1): 241-242.
7. Kumar S., Kumar C.,Phytoconstituents and pharmacological activities of *Musa paradisiaca* Linn, Asian Journal of Biochemical and Pharmaceutical Research, 2012,4(2): 199-206.
8. Banik G., Bawari M., ChoudhuryM.D., Choudhury S. and Sharma, Some anti-diabetic plants of Southern Assam. Assam University, Journal of Science & Technology, Biological and Environmental Sciences, 2010, 5(1): 114-119.
9. T Mudoi, D C Dekaand R Devi,In vitro antioxidant activity of *Garcinia pedunculata*, an indigenous fruitof North Eastern (NE) region of India, International Journal of PharmTech Research, 2012, 4(1): 334-342.
10. Sarma P.C., Ethno antidiabetic plants of Assam, International Journal of Applied Biology and Pharmaceutical Technology, 2011, 2(4): 246-251.
11. Tarak D., Namsa N.D., An inventory of the ethnobotanicals used as antidiabetic by a rural community of Dhemaji district of Assam, Northeast India,Journal of Ethnopharmacology, 2011, 18, 138: 345-350.
12. Nourmohammadi I., Shalmani I.K., Shaabani M., Ladan G., Hossein N., Zinc, copper, chromium, manganese and magnesium levels in serum and hair of insulin-dependent diabetics, Archives of Iranian Medicine, 2000, 3(3): 88-100.
13. Candilish D.J., Minerals, Journal of the American College of Nutrition, 2000, 17: 286-310.
14. Al-Awadi F.M., Anim J.T., Srikumar T.S., Possible role of trace elements in the hypoglycemic effect of plants extract in diabetic rats, The Journal of Trace Elements in Experimental Medicine,2004:1731–1744.
15. Arika W.M., Ogola P.E., Nyamai D.W., Mawia A.M., Wambua F.K., Kiboi N.G., Wambani J.R., Njagi S.M., Rachuonyo H.O., Emmah K.O., Lagat R.C., Muruthi C.W., Abdirahman Y.A., Agyirifo D.S.,Ouko R.O., NgugiM.P.andNjagiE.N.M.,Mineral elements content of selected Kenyan antidiabetic medicinal plants, Advanced Techniques in Biology & Medicine, 2016, 4(1): 2-5.
16. Kanungo S., Mahapatra T., Bhowmik K.,MahapatraS., Saha J., Pal D., Sarkar K., Diabetes scenario in a backward rural district population of India andneed for restructuring of health care delivery services, Epidemiol, 2016, 6(2): 1-10.
17. Kasali F.M., Wendo F.M., Muyisa S.K., NtokamundaKadima J., Comparative hypoglycemic activity of flavonoids and tannins fractions of *Stachytarphetaindica* (L.) Vahl leaves extracts in Guinea-Pig and Rabbits, International, International Journal of Pharmacy and Pharmaceutical Research (Ijppr), 2016, 5(2): 48-57.
18. Mohan, V., Sandeep, S., Deepa, R., Shah, B. and Varghese, C., Epidemiology of type-2 diabetes: Indian scenario. Indian J. Med. Res., 2007, 125: 217–230.
19. Grover J.K., Vats, V., Shifting Paradigm from conventional to alternate medicine, An introduction on traditional Indian medicine, Asia Pacific Biotechnology news, 2001, 5(10): 28-32.
20. Vincent J.B., Quest for the molecular mechanism of chromium action and its relationship to diabete, Nutrition Reviews, 2000, 58(3): 67-72.
21. Kruse-Jarres J.D., Rukgauer M., Trace elements in diabetes mellitus. Peculiarities and clinical validity of determinations in blood cells, Journal of Trace Elements in Medicine and Biology, 2000, 14: 21-27.
22. Srinivasu C.C., Babu N.G., Raju T.P., Narayana P.V.L., Ram S.S., Sudershan M. and Das N.L., Concentration of trace elements in selected Indian antidiabetic medicinal plants by using Edxrf technique, International Journal of Recent Scientific Research, 2016, 7(2): 9104-9108.
23. Pittas A.G., Lau J., Hu F.B., Dawson-Hughes B., The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis, Journal of Clinical Endocrinology and Metabolism, 2007, 92(6): 2017-2029.
24. Chatterjee R., Yeh H.C., Shafi T., Selvin E., Anderson C., Miller E., Brancati F., Serum and dietary potassium and risk of incident type 2 diabetes mellitus: The Atherosclerosis Risk in Communities (ARIC) study, Archives of Internal Medicine, 2010, 170(19): 1745-1751.
25. Rajendra A., Narayan V., Granavel I., Study on the analysis of Trace elements in *Aloe Vera* and its biological importance, Journal of applied sciences Research, 2007, 3(11): 1476-1478.
26. Isbir T., Tamer L., Taylor A., Isbir M., Zinc, copper and magnesium status in insulin-dependent diabetes, Diabetes Res, 1994, 26(1): 41-45.

27. Diwan A.G., Pradhan A.B., Lingojwar D., Krishna K.K., Singh P. *et al*., Zinc, chromium and magnesium levels in type-2 diabetes, International Journal of Diabetes in Developing Countries, 2006, 26(3): 122-123.
28. Chausmer A.B., Zinc, insulin and diabetes, Journal of the American College of Nutrition,1998, 17: 109-14.
29. Tapiero H., Tew K.D., Trace elements in human physiology and pathology: zinc and metallothioneins, Biomed Pharmacother, 2003, 57: 399-411.
30. Mooradian A.D., Morley J.E, Micronutrient status in diabetes mellitus, American Journal of Clinical Nutrition, 1987, 45(5): 877-895.
31. Ngugi M.P., Njagi J., Kibiti C., Maina D., Ngeranwa J. *et al*., Trace elements content of selected Kenyan anti-diabetic medicinal plants, International Journal of Current Pharmaceutical Research, 2012, 4: 39-42.
32. Siddiqui K., Bawazeer N., Joy S.S., Variation in macro and trace elements in progression of type 2 diabetes, Scientific World Journal, 2014: 461591.
33. Zheng Y., Wang Y., Cai L., The role of zinc, copper and iron in the pathogenesis of diabetes and diabetic complications: therapeutic effects by chelators, Hemoglobin, 2008: 32135-145.
34. Wilson J.G., Lindquist J.H., Grambow S.C., Crook E.D., Maher J.F., Potential role of increased iron stores in diabetes, American Journal of Medical Sciences, 2003, 325(6): 332-339.

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