Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

Evaluation of in-vitro Antioxidant and Antidiabetic Activities of *Clematis montana* Ethyl Acetate Leaves Extract

Hemlata Bhatt [1], Bhawana Sati [2*], Lalatendu Mohanty[3] and Prakash Chandra Bhatt[4]

[1,3] Department of Pharmaceutical Sciences, H.N.B. Garhwal University, Srinagar, Garhwal, Uttarakhand, India
[2*] Department of Pharmacy, Banasthali Vidyapeeth, , Rajasthan, India
[4] Indian Medicines Pharmaceutical Corporation Ltd. Mohan, Almora, Uttarakhand, India

Abstract:

Clematis montana belonging to the family *Ranunculacea*, in Garhwal region it is known as Himalayan clematis, but it also available in other himalaya's belt like Kumaoun region in Uttarrakhand, China, Pakistan etc. mainly 1,500–2,000 m above sea level. In Uttarakhand local people used for the treatment of skin related problem, liver disease, headache and high blood pressure.This study was mainly done to findout the *in-vitro* antioxidant and antidiabetic activity of the of *C. montana* leavesethyl acetate extract . The dried powdder of leaves were extracted by using ethyl acetate as a solvent, and then extract was used for *in-vitro* antioxidant assays, by using DPPH radical scavenging, ferrous ion chelating, and reducing power assays methods. α-amylase and α-glucosidase inhibitory assays methods were used to evaluate *in-vitro* antidiabetic activity. The extract showed very promising antioxidant and antidiabetic activities, the results support that *C. montana* leaves have number of bioactive compounds that may responsible for the treatment of oxidative stress and diabetes but for the confirmation of those activities further detailed phytochemical screenings and *in-vivo models* studies are required.

Keywords: *Clematis* ; Ranunculaceae; antioxidant; antidiabetic; DPPH; α-amylase inhibition; α-glucosidase inhibition.

Corresponding author*:* E-mail* bhawanasati28@gmail.com

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

Introduction

At present scenario many health issues are arises, out of those Diabetes mellitus (DM) is a one of the most common disease which is mainly identified by high blood glucose level due to lack of insulin secretion, insulin resistance, or both. The other common symptoms are weakness,hunger, thrust, decrease the body weight etc. In 2016 as per WHO 422 million peoples suffering from Diabetes mellitus all over the world, and 65 million in India only expected to be affected by 2025. The major causes are motionless lifestyle, unhealthy dietary habits, obesity etc. are among the major risk factors which increasing the number of diabetes in India (Akram T Kharroubi et al., 2015, Craig ME, 2009, Galtier F., 2010).

Diabetes mellitus mainly affecting our cardiovascular, nervous, renal, and urinary systems, thus it increasing the mortality rate (S.M. Sadikot et al., 2004). Diabetes and its related complications mainly aggravated due to the oxidative stress (Fisher-Wellman K et al., 2009). Higher concentration of glucose molecules support the formation of reactive oxygen species via different mechanisms, like glucose oxidation, protein and lipid glycation, and sorbitol–aldose reductase pathway (Joshua A et al., 2006). Generally large amount of reactive oxygen species decrease the internal natural anti-oxidant substances like Vitamin C, glutathione etc. and it causes damage of molecules like DNA, RNA, proteins, enzymes, and membrane lipids. If Diabetes remains untreated for longer time it may damage kidney, heart, liver like organ. (Fusun Oztay et al., 2015; Aydın et al., 2001).

In all over the world, formulation of natural remedies foe the treatment and management of Diabetes mellitus is going on, to reduce the long term side effects of synthetic drugs.

Although insulin therapy and oral hypoglycemic agents remain the primary approaches for diabetes management, their long-term use is often associated with adverse effects and limited efficacy in preventing diabetic complications. Therefore, there is a growing interest in

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

identifying safer and more effective antidiabetic agents from medicinal plants (Ratnakar and Murthy, 1996, Ratnakar et al., 1996).

The genus *Clematis* comes under the family Ranunculaceae and consist of more than 200 species. Clematis is also very well known for their ornamental and medicinal value in Uttarakhand. *Clematis montana* commonly known as Himalayan clematis, it is distributed all over the Garhwal Himalaya regions of Uttarakhand and nearby other Himalayan regions. This plant has been used by uttarakhand peoples for the basic treatment of migraine, diabetes, nervous disorders, skin infections, liver disorders, and hypertension (Sastri, 1962; Dhar et al., 1968). Isolation of the triterpenoids bisglycosides and saponins from the *C. montana*, support the anti-oxidant and anti-diabetic activity. (Bahuguna et al., 1990; Thapliyal and Bahuguna, 1993a; 1993b). But the *C. montana* leaves antioxidant and antidiabetic potential remains limited. Hence , our present study was undertaken to find out the *in-vitro* antioxidant (DPPH radical scavenging, ferrous ion chelating, reducing power) and antidiabetic activities (α-amylase inhibitory, and α-glucosidase inhibitory assays) of the ethyl acetate extract of *C. montana* leaves.

Materials and methods

Plant Material Collection and Authentication

Clematis montana leaves were collected from their natural habitat in the Uttarakhand region of India (30.45° N, 78.25° E; altitude: 2,286 m above sea level) during July–August 2012. The plant material was authenticated by Dr. J. K. Tiwari, Department of Botany, H.N.B. Garhwal University, Srinagar Garhwal, Uttarakhand, India, by comparison with the herbarium specimen (GUH 1023) deposited in the Garhwal University Herbarium. A voucher specimen was deposited in the Herbarium of the Department of Pharmaceutical Chemistry, H.N.B. Garhwal University, for future reference. The collected leaves were air-dried in the dark at room temperature (25–30 °C) for 20 days. The dried material was then powdered using a mechanical grinder and stored in airtight containers at room temperature until further use.

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

Preparation of Plant Extract

The powdered leaf material (100 g) was extracted with ethyl acetate using a Soxhlet apparatus for 72 h. The solvent was removed from the extract by distillation under reduced pressure using a rotary evaporator. The concentrated extract was further evaporated to dryness to obtain a crude ethyl acetate extract. The percentage yield of the dried extract was found to be 7.29% (w/w)**.**

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the ethyl acetate extract of *Clematis montana* leaves was carried out using standard qualitative chemical tests to determine the presence of various classes of phytochemical constituents according to the method described by Kokate (2015).

Evaluation of i*n-vitro* antioxidant studies

i) **DPPH Free Radical Scavenging Assay**

The free radical scavenging activity of the ethyl acetate extract of *Clematis montana* leaves was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the method described by Aktumsek et al. (2013). Briefly, 2 mL of DPPH solution (75 μM) was added to 2 mL of the plant extract at various concentrations and to the standard ascorbic acid solution. The reaction mixtures were thoroughly mixed and incubated in the dark for 30 min at room temperature.The control solution was prepared by mixing 2 mL of methanol with 2 mL of DPPH solution. After incubation, the absorbance of the reaction mixtures and the control was measured at 517 nm using a UV–Visible spectrophotometer.

The percentage inhibition of DPPH radicals was calculated using the appropriate equation. A graph was plotted between percentage inhibition (%) and the different concentrations of the plant extract and ascorbic acid. From the graph, the IC_{50} value was determined. The IC_{50} value represents the concentration of the sample required to scavenge 50% of the DPPH free radicals.

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

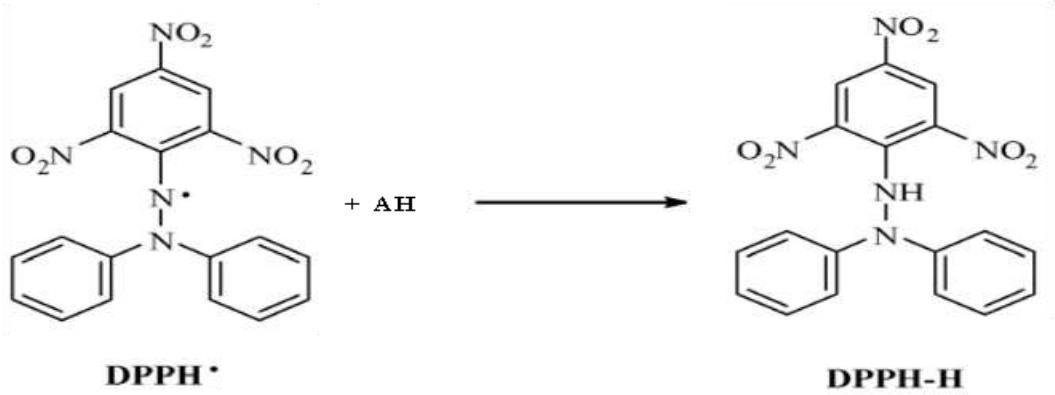

Fig.1. Reaction of 1,1-diphenyl-2- picryl-hydrazyl free radical with an antioxidant

ii) Reducing power assay

The reducing power of the ethyl acetate extract of *Clematis montana* leaves was evaluated according to the method described by Sasikumar et al. (2012). Briefly, 1 mL of each concentration of the plant extract, the standard, and the control (methanol) was mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide solution. The reaction mixtures were thoroughly mixed and incubated at 50 °C for 20 min. After incubation, the mixtures were cooled to room temperature, and 2.5 mL of 10% (w/v) trichloroacetic acid was added. The mixtures were then centrifuged at 3,000 rpm for 10 min.Following centrifugation, 2.5 mL of the supernatant was mixed with an equal volume of distilled water, and 0.5 mL of 0.1% (w/v) ferric chloride solution was added. The reaction mixtures were thoroughly mixed and allowed to stand at room temperature for 10 min. The absorbance was measured at 700 nm using a UV–Visible spectrophotometer.

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

iii) Ferrous chelating activity

The ferrous chelating activity of ethyl acetate extract of *C. montana* was evaluated according to the method given by FU, 2012 (FU et al., 2012). To 1 ml each of various concentrations of extracts and standard EDTA as well as control (methanol), ferrous chloride (0.2 ml, 2mM) and ferrozine (0.4 ml, 5mM) were added. The desired volume of 4 ml was made by adding methanol. It allowed to stand for 10 min at room temperature after the shaken of reaction mixture. The absorbance of all the reaction mixtures was recorded at 562 nm. The ferrous chelating activity was determined by using following formula:

$$\text{Chelating activity (\%)} = (A_0 - A_1)/ A_0 \times 100$$

Where A_0 represents the absorbance of control and A_1 represents the absorbance of plant extracts or standard.

Evaluation of in-vitro anti-diabetic activity

i) α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity of the ethyl acetate extract of *Clematis montana* leaves was evaluated according to the methods described by Kumar et al. (2011), Matsui et al. (1996), and Kang et al. (2011). Briefly, the reaction mixture consisted of 2.9 mM 4-nitrophenyl-β-D-glucopyranoside (pNPG) as the substrate, 0.25 mL of the test sample, and α-glucosidase (6 U/mL) prepared in sodium phosphate buffer (pH 6.9). The reaction mixture, along with the corresponding blank, was incubated at 25 °C for 5 min. After incubation, the absorbance was measured at 405 nm using a UV–Visible spectrophotometer.

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

ii) α-Amylase Inhibitory Activity

The α-amylase inhibitory activity of the ethyl acetate extract of *Clematis montana* leaves was determined according to the method described by Kwon et al. (2007), with slight modifications from Hansawasdi et al. (2000) and Ranilla et al. (2010). Briefly, 2 mg of starch azure was suspended in 0.2 mL of 0.5 M Tris-HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ to prepare the substrate solution. The suspension was boiled for 5 min and subsequently incubated at 37 °C for 5 min.

Thereafter, 0.2 mL of the test sample and 0.1 mL of pancreatic α-amylase solution (2 U/mL prepared in Tris-HCl buffer) were added to the substrate solution. The reaction mixture was incubated at 37 °C for 10 min. The reaction was terminated by adding 0.5 mL of 50% (v/v) acetic acid. The mixture was then centrifuged at 3,000 rpm for 5 min at 4 °C, and the supernatant was collected. The absorbance of the supernatant was measured at 595 nm using a UV–Visible spectrophotometer. Acarbose was used as the standard reference drug.

Results and discussion

Preliminary phytochemical screening

Preliminary phytochemical screening of the ethyl acetate extract of *Clematis montana* leaves revealed the presence of carbohydrates, tannins, steroids, triterpenoids, saponins, and flavonoids (Table 1).

Table:1. Phytochemical screening of *C. montana* leaves ethyl acetate extract

S. No.	Test	Leaves
1	Saponines	+
2	Triterpenoids	+
3	Tannins	++
4	Flavonoids	++

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

5	Steroids and sterols	++
6	Carbohydrates	+
7	Alkaloids	-

The saponins presence in the ethyl acetate extract of *Clematis montana* suggests that they may contribute to its pharmacological activities. Previous studies have reported that saponins isolated from *Clematis* species possess significant cytotoxic, anti-inflammatory, antidiabetic, diuretic, and antifungal properties (Bodakhe et al., 2009; Bernela et al., 2016). Likewise, flavonoids present in *Clematis* species have been reported to exhibit analgesic, anti-inflammatory, and antipyretic activities. In addition, the presence of phenolic compounds in the extract may be responsible for its antioxidant activity. Phenolic compounds are known to possess diverse biological properties, including antioxidant, anti-inflammatory, antidiabetic, and anticancer activities, and may therefore play an important role in the prevention and management of various diseases.

Antioxidant activity (*in-vitro*)

i) Free radicals scavenging activity (DPPH)

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical that exhibits a deep violet color with a characteristic absorption maximum at 517 nm. The basic principle involved in this method is that When antioxidant mix on the DPPH solution, the antioxidant molecules release a hydrogen atom or an electron to the DPPH radical. Due to this reaction the color of the DPPH solution is change from deep violet to yellow.

In this study, the *Clematis montana* leaves ethyl acetate extract (CMLEAE) indicated concentration-dependent anti-oxidant activity. CMFEAE showed maximum percentage inhibition at a 500 µg/mL, which is 79.87% but less than standard ascorbic acid 80.22% at a 10

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

µg/mL. The IC_{50} values of ascorbic acid was 6.727 µg/mL and for CMLEAE it was112.93 µg/mL. The antioxidant potency of CMLEAE may be due to the presence of phenolic compounds, flavonoids etc. like phytochemicals.

Table : 2. **CMLEAE and standard ascorbic acid IC_{50} value by DPPH method**

S. No.	EXTRACT		ASCORBIC ACID	
	Concentration (µg/ml)	**% Inhibition**	**Concentration (µg/ml)**	**% Inhibition**
1	100	48.91	2	13.22
2	200	57.39	4	23.42
3	300	63.56	6	43.24
4	400	70.57	8	59.04
5	500	79.87	10	80.22
	IC 50	**112.93µg/ ml**	**IC 50**	**6.727 µg/ ml**

ii) Ferrous chelating activity

The basic principle of assay is, ferrozine forms complex with ferrous ions (magenta color). But the anti-oxidant molecules competes with ferrous ions to form the complex with ferrozine due to this intensity of magenda color decrease. As intensity of magenda color decreases absorbance also decreases. Thus, the chelating activity of the CMLEAE was analyzed by measuring the decrease in absorbance of the ferrozine–Fe^{2+} complex.

Clematis montana leaves (CMLEAE) showed concentration-dependent increase in the ferrous chelating activity. As the concentration of the extract increased, the intensity of magenda color decreased so absorbance also decreased, this result showing the increased metal-chelating activity. The results are shown in Table 3. The IC_{50} value of CMLEAE was 673.78 µg/mL and

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

the IC_{50} value of standard EDTA was 37.65 µg/mL. This chelating activity of the extract was less than EDTA, but the extract revealed significant ferrous chelating activity.

Table :3. CMLEAE and EDTA IC_{50} value by ferrous chelating method

S. No.	EXTRACT		EDTA	
	Concentration (µg/ml)	% Inhibition	Concentration (µg/ml)	% Inhibition
1	200	31.56	10	10.4
2	400	39.67	20	26.45
3	600	49.95	30	40.79
4	800	55.08	40	53.23
5	1000	61.22	50	66.2
	IC 50	**673.78 µg/ ml**	**IC 50**	**37.65 µg/mL**

iii) Reducing power assay

At a concentration of 500 µg/mL the ethyl acetate extract of *Clematis montana* leaves (CMLEAE) showed maximum absorbance. The absorbance of standard was 1.12 and CMLEAE was 0.908, which was lower than the standard value. Further the IC_{50} values of standard and extract were 43.7 µg/mLand 250.35 µg/mL , respectively. These values indicate that extract has significant anti-oxidant activity but less than ascorbic acid.

All the above mentioned results support the review regarding ant-oxidant activity of Clematis but further isolation of molecules from the plant and their cellular level mechanism studies are required in future.

Table: 4. IC_{50} value of CMLEAE and standard ascorbic acid by reducing power assay

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

S. No.	EXTRACT		ASCORBIC ACID	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	100	0.202	20	0.282
2	200	0.465	40	0.48
3	300	0.589	60	0.672
4	400	0.743	80	0.884
5	500	0.908	100	1.12
	IC 50	**250.35 µg/ ml**	**IC 50**	**43.7 µg/ ml**

4.6 *In- vitro* antidiabetic activity

i) α-Glucosidase inhibitory activity

α-Glucosidase is a key digestive enzyme that catalyzes the hydrolysis of oligosaccharides and disaccharides into absorbable monosaccharides in the small intestine. Inhibition of this enzyme delays carbohydrate digestion and glucose absorption, thereby reducing postprandial hyperglycemia. Clinically used α-glucosidase inhibitors, such as acarbose, miglitol, and voglibose, are effective in controlling postprandial blood glucose levels in patients with diabetes mellitus.

The α-glucosidase inhibitory activity of the ethyl acetate extract of *Clematis montana* leaves (CMLEAE) is presented in Table 5. CMLEAE exhibited concentration-dependent α-glucosidase inhibitory activity, with a maximum inhibition of 78.60 ± 0.66% at a concentration of 90 µg/mL. In comparison, the standard drug acarbose showed 96.19 ± 0.91% inhibition at the same concentration. The IC_{50} values of CMLEAE and acarbose were 41.56 µg/mL and 30.94 µg/mL, respectively.

The α-glucosidase inhibitory activity of extract was lower in comparison to standard acarbose. The α-glucosidase inhibitory activity of extrac support the presence of different types of natural

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

molecules present in *Clematis* like flavonoids, phenolic compounds, saponins, and triterpenoids etc. which are already have enzyme inhibitory activities. Thus, future studies are required to evaluate the exact mechanisms of action plant moieties.

Table 5: In-vitro α- glucosidase inhibitory activity of CMLEAE and acarbose

Sample	Concentration(µg/ml)	% inhibition	IC50 (µg/ml)
CMLEAE	10	31.21±0.51	41.56
	30	45.69±0.92	
	50	53.72±0.46	
	70	65.07±0.83	
	90	78.60±0.66	
Acarbose	10	38.03±0.83	30.94
	30	47.22±2.32	
	50	61.76±0.65	
	70	75.89±0.78	
	90	96.19±0.91	

ii) α-Amylase Inhibition

α-Amylase inhibitors play an important role in controlling postprandial hyperglycemia by delaying the digestion of dietary starch and the subsequent absorption of glucose. α-Amylase is a key digestive enzyme secreted by the pancreas that catalyzes The conversion of polysaccharides molecules (starch into monosaccharides molecules (glucose) . So the α-amylase inhibition decreases the glucose release and its absorption, by this mechanism it helpful to maintain the glucose level in the blood after the meal. Acarbose, is used as a standard α-amylase inhibitor in our study.

The result showed concentration-dependent α-amylase inhibitory activity of the extract (highest percentage inhibition 64.79 ± 0.29% at a 90 µg/mL) and the standard acarbose showed 79.82 ± 1.65% inhibition at 90 µg/mL. The results are represented in table 6.

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

The CMLEAE shown significant α-amylase inhibitory activity but it was less than acarbose. The data showed that the plant extract has enzyme inhibitory activity, but further investigation required in future to investigate the mechanism of action.

Table 6: In-vitro α- amylase inhibitory activity of CMLEAE and acarbose

Sample	Concentration(µg/ml)	% inhibition	IC 50 (µg/ml)
CMLEAE	10	23.7±0.97	61.32
	30	33.59±0.46	
	50	46.21±1.43	
	70	53.07±0.37	
	90	64.79±0.29	
Acarbose	10	26.19±0.83	46.78
	30	36.57±0.77	
	50	52.9±0.49	
	70	65.61±0.38	
	90	79.82±1.65	

Conclusion

The *Clematis montana* plant used by local peoples for different health related issues, out of them one is diabetes. Our ethyl acetate extract of the leaves support the review related of plant *Clematis montana. This plant extract have significant anti-oxidant and anti-diabetic value, which was proven by DPPH free radical scavenging activity, reducing assay, ferrous chelating activity, and enzyme inhibition (* α-amylase and α-glucosidase). The anti-oxidant and anti-diabetic activity showed by extract of the plant may be the presence of phenolic compounds, flavonoids, saponins, and triterpenoids. In future further studies are required to prove mechanism of action of constituents which are responsible for those types of activity. In conclusion we can say that *Clematis montana* have significant anti-oxidant and anti-diabetic activity.

Acknowledgements

We, the authors are thankful to H.N.B. Garhwal, University Srinagar, Garhwal, Uttarakhand, India for providing facilities and encouragement.

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

References

1. Aktumsek A, Zengin G, Guler GO, Cakmak YS, Duran A., 2013. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. *Food Chem Toxicol*, Vol. 55, pp. 290-6.
2. Akram T Kharroubi, Hisham M Darwish, Diabetes mellitus: The epedemic of the centuary, World J Diabetes, 2015 Jun 25;6(6):850–867.
3. Atalay, M. and Laaksonen, D.E., 2002. Diabetes, oxidative stress and physical exercise. *Journal of Sports Science & Medicine*, Vol. 1, pp. 1-14.
4. Aydın, A., Orhan, H., Sayal, A., Özata, M., Şahin, G. and Işımer, A., 2001. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. *Clinical Biochemistry*, Vol. 34, pp. 65-70.
5. Bahuguna R.P, Thapliyal R.P, Murakami N, Tanase T, Kaiya T, Sakakibara J, 1990. Saponins from *Clematis Montana. Pharmaceutical Biology,* Vol. 28, issue 2, pp. 125-7.
6. Bernela Manju, Munish Ahuja, Rajesh Thakur, 2016. Enhancement of anti-inflammatory activity of glycyrrhizic acid by encapsulation in chitosan-katira gum nanoparticles. *Journal of Pharmaceutics and Biopharmceutics*, Vol. 105, pp. 141–147
7. Bodakhe, S.H.; Ram, A.; Bodakhe, K.S., 2009. Anti-inflammatory activity of Morina longifolia extract. *Advances in Pharmacology and Toxicology*, Vol. 2, Issue 10, pp. 71-79.
8. Craig ME, Hattersley A, Donaghue KC. Definition, epidemiology and classification of diabetes in children and adolescents. Pediatr Diabetes. 2009;10 Suppl 12:3–12.
9. Dhar M.L, Dhar M.C, Dhawan B.N, Mehtrotra B.N and Ray C., 1968. Screening of Indian plants for biological activity: Part 1. *Indian Journal of Experimental Biol*ogy, Vol.2**,** pp. 232-47.
10. Eidi A., 2006. Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin induced diabetic rats. *Phytomedicine*, Vol. 13, pp. 624-9.
11. Fisher-Wellman K, Bell HK, Bloomer RJ.Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders. Oxid Med Cell Longev. 2009 Jan-Mar;2(1):43-51.

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

12. FU M, Feng H, Chen Y,Wang D, Yang G., 2012. Antioxidant activity of *Garcinia xanthochymus* leaf, root and fruit extracts in vitro. *Chinese Journal of Natural Medicines*, Vol.10, Issue 2, pp.129−34.
13. Fusun Oztay, Ozlem Sacan, Ozgecan Kayalar, Sehnaz Bolkent, Yesim Ipci, Levent Kabasakal, Goksel Sener and Refiye Yanardag.Chard (*Beta vulgaris* var. *cicla*) extract improved hyperglycemia-induced oxidative stress and surfactant-associated protein alterations in rat lungs.Pharmaceutical Biology, Volume 53, 2015 - Issue 11.
14. Galtier F. Definition, epidemiology, risk factors. Diabetes Metab. 2010;36:628–651.
15. Gumieniczek, A., Hopkala, H., Wojtowicz, Z. and Nikolajuk, J., 2002. Changes in antioxidant status of heart muscle tissue in experimental diabetes in rabbits. *Acta Biochemica Polonica.* Vol.49, Issue 2, pp.529-535.
16. Hansawasdi, C., Kawabata, J., Kasai, T., 2000. Alpha-amylase inhibitors from roselle (*Hibiscus sabdariffa* Linn.) tea. *Bioscience, Biotechnology, and Biochemistry*, Vol. 64, pp. 1041-3.
17. Joshua A. Scott, George L. King. Oxidative Stress and Antioxidant Treatment in Diabetes, Annals of the New York Acadamy of the Sciences, 12 January 2006.
18. Kang, W.Y., Song, Y.L., Zhang, L., 2011. α-Glucosidase inhibitory and antioxidant properties and antidiabetic activity of *Hypericum ascyron* L. *Medicinal Chemistry Ressearch* ,Vol. 20, pp. 809-816.
19. Kokate, C. K., Purohit A. P., Gokhale, S. B., 2006. *Practical Pharmacognosy*. 4th edn., vallabh Prakashan, Delhi, pp. 107-108.
20. Kumar, S., Narwal, S., Kumar, V., Prakash, O., 2001. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Review*, Vol. 5, pp.19-29.
21. Kwon, Y.I., Apostolidis, E., Kim, Y.C., Shetty, K., 2007. Health benefits of traditional corn, beans and pumpkin: *In vitro* studies for hyperglycemia and hypertension management. *Journal of Medicinal Food*, Vol. 10, pp. 266-75.
22. Ranilla, L.G., Kwon, Y.I., Apostolidis, E., Shetty, K., 2010. Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for

ISSN: 0975-3583,0976-2833 VOL 9, ISSUE 3, 2018

hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource Technology*, Vol.101, pp. 4676-89.

23. Ratnakar P, Murthy PS, 1996. On mechanism of action of water extract of garlic and two partially purified proteins. *Indian Journal of Clinical Biochemistry*, Vol. 11, pp. 97-41.
24. Sasikumar JM, Patharaj J, Adithya ES, Christabel PH, Shamna R., 2012. Antioxidant capacity and phenolic content of *Elaeagnus kologa schlecht*. An underexploited fruit from India. *Free Radicals and Antioxidants*, Vol.2, Issue 3, pp. 28-35.
25. Sastri B.N., 1962. *The Wealth of India*. Publication and Information Directorate, CSIR. Hillside, New Delhi, India, pp. 336.
26. Satyanarayana T, 2006. Hypoglycemic and antihyperglycemic effect of alcoholic extract of Euphorbia leucophylla and its fractions in normal and in alloxan induced diabetic rats. *Pharmacognosy Magazine*,Vol. 2, pp. 244-53.
27. Shaw JE, Sicree RA, Zimmet PZ, 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, Vol. 87, pp. 4-14.
28. S.M. Sadikot, A. Nigam, S. Das, S. Bajaj, A.H. Zargar, K.M. Prasannakumar, A. Sosale, C. Munichoodappa, V. Seshiah, S.K. Singh, A. Jamal, K. Sai, Y. Sadasivrao, S.S. Murthy, D.K. Hazra, S. Jain, S . Mukherjee, S. Bandyopadhay, N.K. Sinha, R. Mishra…K. Goenka. The burden of diabetes and impaired fasting glucose in India using the ADA 1997 criteria: prevalence of diabetes in India study (PODIS). Diabetes Research and Clinical Practice, Volume 66, Issue 3, December 2004, Pages 293-300.
29. Thapliyal RP, Bahuguna RP, 1993. Clemontanoside-C. A saponin from *Clematis montana*. *Phytochemistry*, Vol. 33, Issue 3, pp.671-3.
30. Thapliyal RP, Bahuguna RP, 1993. An oleanolic acid based bisglycoside from *Clematis montana* roots. *Phytochemistry*, Vol. 34, Issue 3, pp.861-2.
31. West, I.C., 2000. Radicals and oxidative stress in diabetes. *Diabetic Medicine*, Vol. 17, pp. 171-180.
32. Zimmet, P.Z., McCarty, D.J. and Courten, M.P., 1997. The global epidemiology of non-insulin dependent diabetes mellitus and the metabolic syndrome. *Journal of Diabetes and its Complication*, Vol.11, pp. 60-68.