www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(1): 61-66

www.thepharmajournal.com
Received: 10-01-2015
Accepted: 17-02-2015

M. Amin Mir
Uttaranchal College of Science and Technology, Dehradun India.

S. S. Sawhney
Uttaranchal College of Science and Technology, Dehradun India.

M. M. S. Jassal
Dept. of Chemistry D. A. V. (P.G) College Dehradun India.

Correspondence:
M. Amin Mir
Uttaranchal College of Science and Technology, Dehradun India.

In-vitro antidiabetic studies of various extracts of *Taraxacum officinale*

M. Amin Mir, S. S. Sawhney, M. M. S. Jassal

Abstract
Diabetes is a common and dangerous disease worldwide and its main factor is the insulin deduction leading to the increment in the blood glucose level, whose consequences lead to various cardiovascular diseases. There are chemical substances which act like insulin but have side effects associated with them. So Ayurvedic systems moved towards the alternative which doesn't bear adverse effects in addition to the insulin property. So this study upon the antidiabetic properties was carried out in which alpha-amylase and alpha glucoside inhibition showed good response. The results showed that water extracts of all plant parts have highest antidiabetic properties as compared to that of methanol extracts. Among the different parts of the plant, the stem showed highest activity fallowed by roots and least activity furnished by flowers of plant.

Keywords: Antidiabetic, *Taraxacum officinale*, *In-vitro*, Alpha-amylase, Alpha-glucosidase

1. Introduction
The plants have been used as a natural cure for several ailments and diseases and are one of the oldest practices by mankind. The plants are being used as imperative medicinal herbs sine times immoral, because in the treatment of disease, it is not the drug used to treat and cure the disease that matters, but to conserve the body mechanism in a regular way is of paramount importance, which is possible only due the usage of herbal medicine Robert F *et al.* [1]. The role of plants as chemicals constituent vessels bearing pharmaceutical potential have been studied from a very long Maurice AE [2].

Taraxacum officinale F. H. Wigg., commonly known as Dandelion (from the French dent-de-lion meaning lion's tooth) is thought to have evolved about thirty million years ago in Eurasia. The chief constituents of dandelion root are taraxacin, taraxacerin, and inulin (a sort of sugar which replaces starch in many of the Dandelion family, Asteraceae), gluten, gum and potash. Dandelions are one of nature's richest green vegetable sources of beta- carotene, from which vitamin A is created (14000 iμ/100 g leaves vs. 11 000 iμ/100 g in carrots). It is an important herb and is versatile in its nature, as being the whole plant can be used for medicinal as well as culinary purposes. Medicinally, dandelion is considered to be as anti-diabetic, detoxicant aperient, diuretic, stomachic, tonic, and stimulant Clarke CB [3]. Forty percent of the mature root is inulin, a mixture of complex carbohydrates known as fructo-oligosaccharides (FOS). Based on clinical studies, intake of FOS significantly increases beneficial bifido-bacteria within the gastrointestinal tract and eliminates pathogens.

Forty percent of the mature root of taraxacum officinale carries inulin, a mixture of complex carbohydrates known as fructo-oligosaccharides (FOS). As per the clinical studies, intake of FOS significantly eliminates pathogens within the gastrointestinal tract due to increase in beneficial bifido-bacteria. Immune system also gets stimulated by FOS due to increase in the mineral absorption and suppression of abnormal cell growth. The high levels of FOS in dandelion plant and in its various water extracts help the body to keep blood sugar levels constant and reduce hyperglycemia.

Diabetes is one of the most fetal major causes of death worldwide. As per reports in every ten second a person dies from diabetes related problems. In 2007, it had been reported that diabetes caused 3.5 million deaths globally Das AK *et al.* [4]. Diabetes is known as 'a disease of rich man' but had now spread among all masses Gupta R *et al.* [5] in India as well in the whole world. Developing countries have large number of diabetic patients and indeed, India has been infamously dubbed as the 'diabetic capital of the world' Abate N *et al.* [6] because of the largest number of diabetic patients in the world.

2. Materials and Methods

2.1 Plant materials

The plant material was collected from Kupwara region of Kashmir and was authenticated from FRI Dehradun. The collection process was preferably done in the dry condition. Plant was weighed before and after the removal of unwanted material kept under shade at room temperature for the removal of extra moisture. The plant samples were air dried and grounded into uniform powder with a grinder. All the plants parts i.e. stem, flowers and roots were collected separately and were subjected to different operations individually.

2.2 Experimental

All the chemicals used in this investigation were of analytical reagent (AR) grade and were purchased from Sigma Merck. De-ionized water was used for the complete study. All the glassware and equipment used for handling were stabilized properly prior to use.

2.3 Extraction

The extraction procedure was carried out first with methanol and then with water based upon their polarity index. The extraction was done by Soxhlet extraction method. A thimble was used in order to get the purest form of extract. 75 g of the root material was used for extraction, 60 g of flower and 95 g of stem plant material was used for extraction purpose. The extraction procedure was carried out separately for different plant parts in a same manner.

2.4 Methodology

***In vitro* methods employed in antidiabetic studies Hamdan [7]**

Inhibition of alpha amylase enzyme

A total of 500 µL of test samples and standard drug (100-1000 µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/ml) solution and were incubated at 25 °C for 10 min. After these, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 di-nitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle Thalapaneni NR *et al.* and Heidari R *et al.* [8, 9].

Inhibition of alpha-glucosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37 °C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35 °C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540 nm Krishnaveni SB *et al.* [10].

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I%) was calculated by

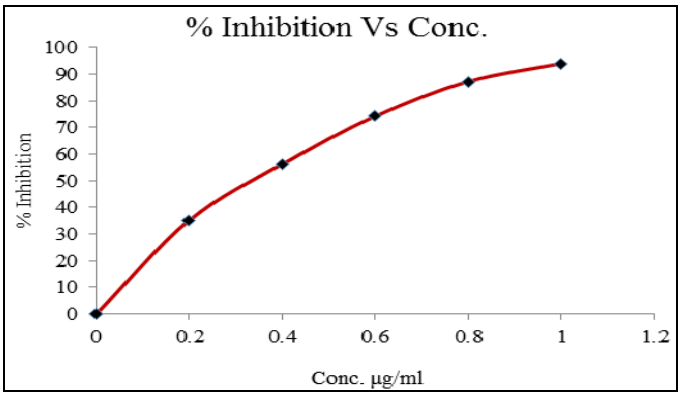
$$I\ \% = (Ac-As)/Ac\ X\ 10 \quad [11]$$

Where Ac is the absorbance of the control and as is the absorbance of the sample.

2.5 Observation

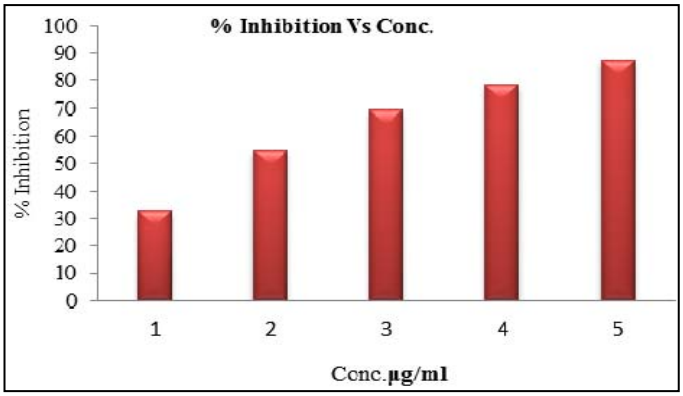
In vitro antidiabetic activity of alpha-amylase method of ACAROSE Standard of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% of Inhibition	IC_{50}
1	00	0.00	**32.55**
2	20	35.12	
3	40	56.22	
4	60	74.23	
5	80	87.22	
6	100	93.97	



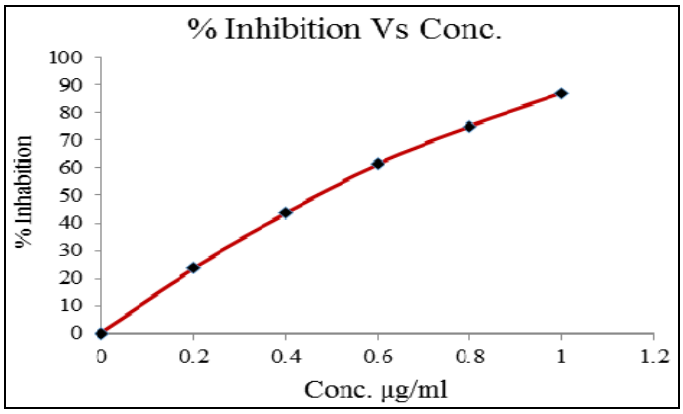
In vitro antidiabetic activity of alpha-amylase method of Water Extract (Stem) of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% of Inhibition	IC 50
1	20	26.8	**43.05**
2	40	47.9	
3	60	60.5	
4	80	85.2	
5	100	94.3	



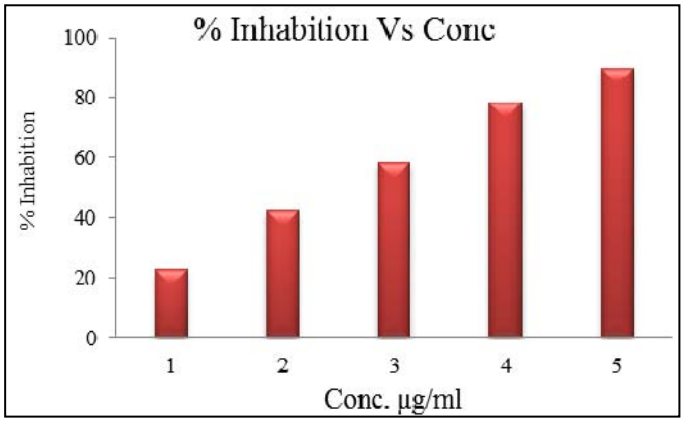
In vitro antidiabetic activity of alpha glucosidase method of Water Extract (Stem) of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% of Inhibition	IC 50
1.	00	00.00	**55.50**
2	20	30.5	
3	40	47.5	
4	60	55.3	
5	80	75.1	
6	100	87.3	



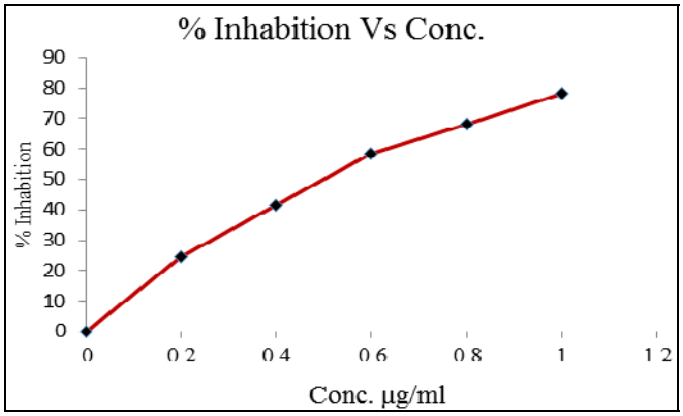
In vitro antidiabetic activity of alpha- amylase method of methanol Extract (Stem) of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% of Inhibition	IC50
1	20	22.8	**47.40**
2	40	41.5	
3	60	60.5	
4	80	77.5	
5	100	85.9	



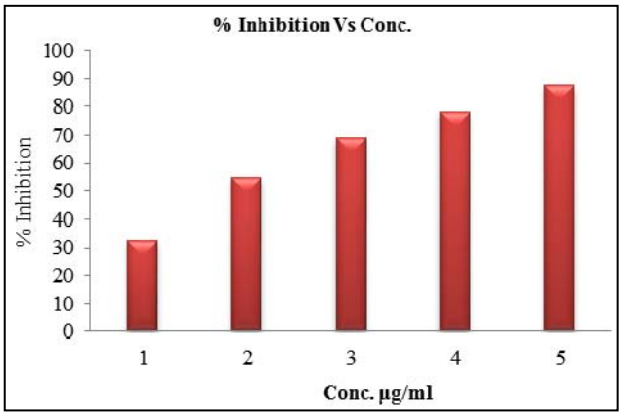
In vitro antidiabetic activity of alpha glucosidase method of methanol Extract (Stem) of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% Of Inhibition	IC50
1	0.00	0.00	**56.77**
2	20	24.5	
3	40	41.5	
4	60	58.3	
5	80	68.1	
6	100	76.3	



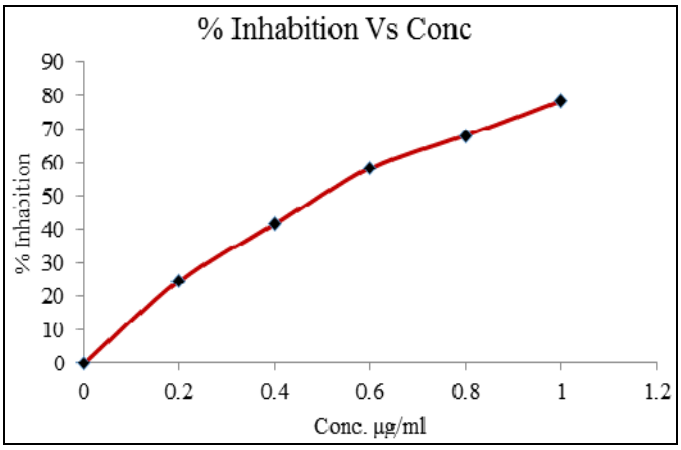
In vitro antidiabetic activity of alpha- amylase method of Water Extract (Root) of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% of Inhibition	IC50
1	20	31.7	**52.00**
2	40	43.9	
3	60	55.2	
4	80	69.5	
5	100	87.4	



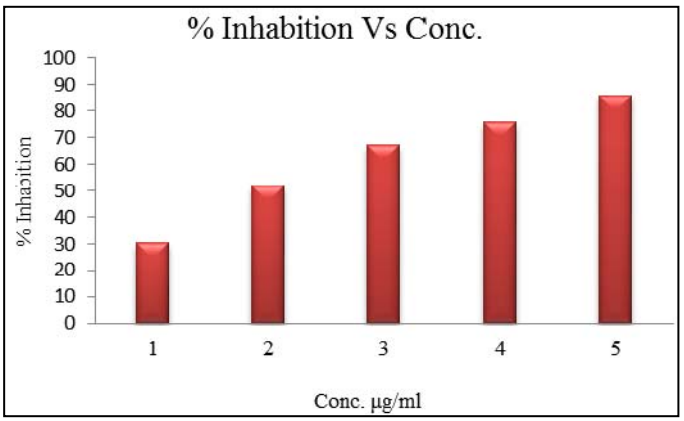
In vitro antidiabetic activity of alpha glucosidase method of Water Extract (Root) of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% Of Inhibition	IC50
1	0.00	0.00	**51.55**
2	20	24.5	
3	40	41.5	
4	60	58.3	
5	80	68.1	
6	100	78.3	



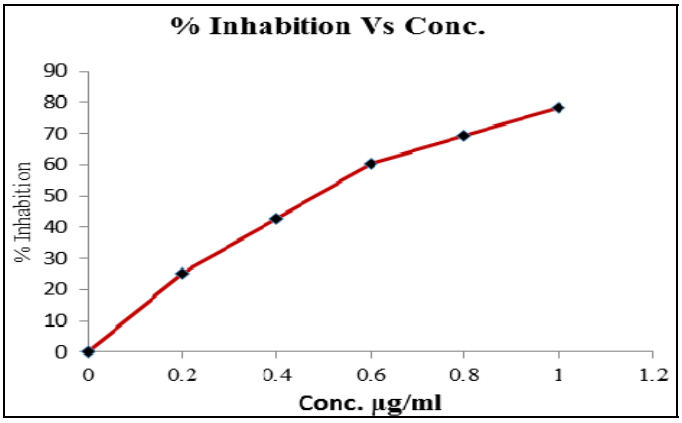
In vitro antidiabetic activity of alpha amylase method of methanol Extract (Root) of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% of Inhibition	IC50
1	20	30.5	**51.75**
2	40	48.8	
3	60	67.1	
4	80	75.6	
5	100	85.9	



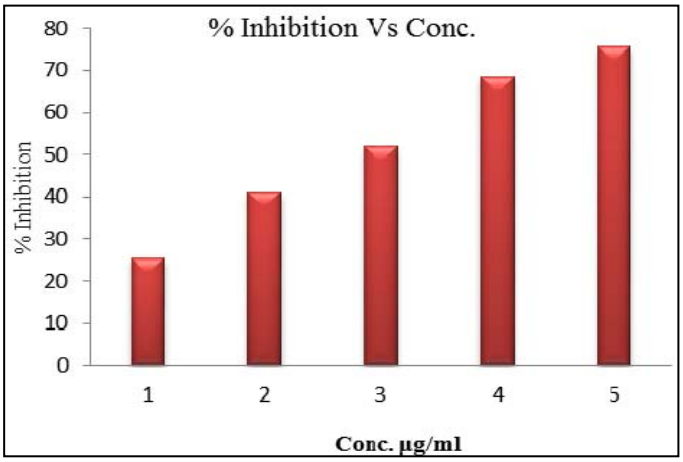
In vitro antidiabetic activity of alpha glucosidase method of methanol Extract (Root) of *Taraxacum officinale*

S. No	Concentration of Sample (µg/ml)	% Of Inhibition	IC_{50}
1	0.00	0.00	**53.20**
2	20	25.1	
3	40	42.7	
4	60	60.3	
5	80	69.5	
6	100	78.1	



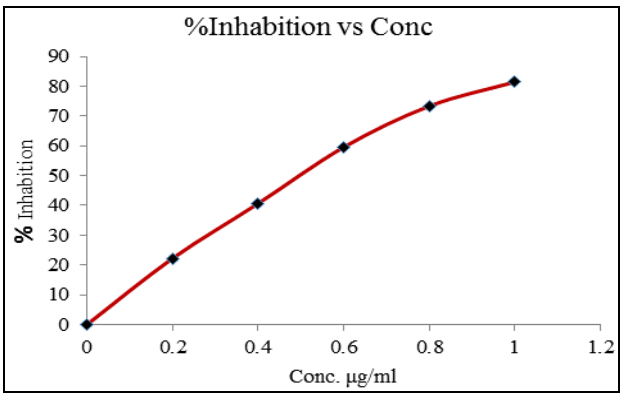
In vitro antidiabetic activity of alpha amylase method of Water (Flower) Extract of *Taraxacum officinale*

S. No	Concentration of Sample (ml)	% of Inhibition	IC_{50}
1	20	25.5	**57.00**
2	40	41.1	
3	60	52.1	
4	80	68.5	
5	100	75.5	



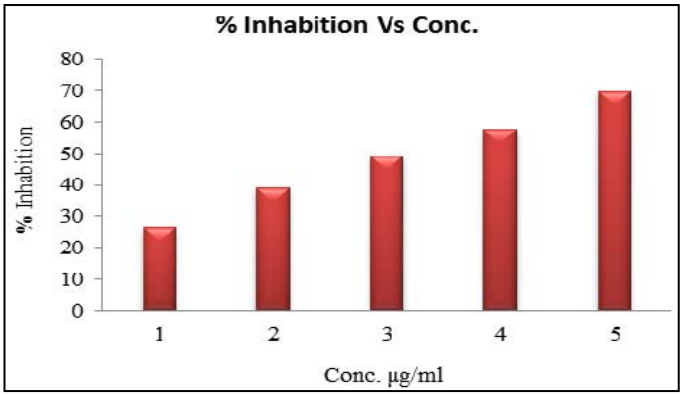
In vitro antidiabetic activity of alpha glucosidase method of Water Extract (Flower) of *Taraxacum officinale*

S. No	Concentration of Sample (ml)	% Of Inhibition	IC_{50}
1	0.00	0.00	**54.35**
2	20	22.2	
3	40	40.6	
4	60	55.5	
5	80	73.3	
6	100	81.4	



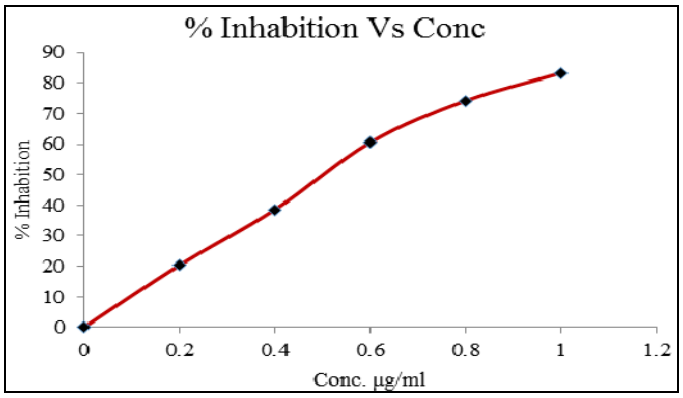
In vitro antidiabetic activity of alpha amylase method of methanol (Flower) Extract of *Taraxacum officinale*

S. No	Concentration of Sample (ml)	% of Inhibition	IC_{50}
1	20	26.1	**61.50**
2	40	39.1	
3	60	49.3	
4	80	57.5	
5	100	69.8	



In vitro antidiabetic activity of alpha glucosidase method methanol (Flower) Extract of *Taraxacum officinale*

S. No	Concentration of Sample (ml)	% Of Inhibition	IC_{50}
1	0.00	0.00	**51.40**
2	20	20.3	
3	40	38.5	
4	60	60.5	
5	80	74.2	
6	100	83.2	



3. Results

As per the results obtained from the respective study it is being shown that there was a dose-dependent increase in percentage inhibitory activity against alpha amylase enzyme. The percentage inhibition value correspondingly increases with increase in the dose concentration as shown in the respective tables and graphs. The water extracts of all the plant parts (Roots, Flower, and Stem) showed marginally more inhibitory effect as compared to the methanol extracts which could be due the more ionic constituents being found in the water extracts than in the methanol extracts.

Among the all parts of the plant the stem showed the highest overall inhibitory effect of both (**alpha amylase + alpha glucosidase)** as an average of about 87.2% fallowed by roots showing nearly 81.7% inhibition, and flowers showed the least inhibitory effect of about 77.47%.

The inhibition percentage of alpha amylase was found to be 85.73% by water extracts and 84.23% by methanol extracts, similarly the percentage inhibition of alpha glucosidase was found to be 78.38% by water extracts and of 79.93% by methanol extracts. In total it is being shown that *Taraxacum officinale* have more profound effect upon alpha amylase enzyme than on the alpha glucoside enzyme.

4. Discussion

Diabetes mellitus is a metabolic disorder and the key factor for its control is insulin. Lack of insulin in the body of an organism affects carbohydrate, fat and protein metabolism Rajiv Gandhi G *et al.* [12]. The control over diabetes mellitus without side effects is a challenge to medical community. Synthetic inhibitor causes side effect such as abdominal pain, diarrhea and soft faeces in the colon. The inhibition of alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which causes a decrease in the absorption of glucose; as a result the elevation of postprandial blood glucose level reduces Rhabaso Lhoret R *et al.* [13].

The inhibitors of Alpha-glucosidase retard the digestion of carbohydrates and slow down the absorption. Acarbose and miglitol are known to be the competitive inhibitor of α-glucosidases and reduces absorption of starch and disaccharides Davis SN *et al.* [14]. Hence the therapeutic approaches for reducing postprandial (PP) blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Postprandial blood glucose level in diabetic patients gets increased due to the inhibition of two of these two enzymes (α-amylase and α-glucosidases) Conforti F *et al.* [15]. The α- amylase inhibitors also act as an anti-nutrients and obstructs the digestion and absorption of carbohydrates. Acarbose as being a complex oligosaccharide delays the digestion of carbohydrates and inhibits the action of pancreatic amylase in breakdown of starch.

In the present study, research has been carried out to evaluate the inhibiting potential of alpha-glucosidase and alpha-amylase. The present finding reveals that *Taraxacum officinale* efficiently inhibits both alpha-amylase and alpha-glucosidase. The water extracts although showed higher inhibition potential than that of methanol, and also it was found that plant responded more towards alpha amylase than that of alpha glucosidase.

5. Conclusion

The present study revealed that methanol and water extracts of *Taraxacum officinale* exhibit considerable α-amylase and α-glucosidase inhibitory activities. Further, this study supports that the concerned plant can be used as an ethnomedicine for management of diabetes.

6. References

1. Robert F, Barnes C, Jerry N, Kenneth JM, Michael C. Forages: The Science of Grassland Agriculture. Wiley-Blackwell, 2007, 11.
2. Maurice AE. Some common locations of Dandelion in Africa. African handbooks, 2003, 12.
3. Clarke CB. Edible and useful plants of California. Berkeley: University of California press, 1997, 191.
4. Das AK, Rai. A world without diabetes and its complications: A preventive program. In: Jayaram BM (Ed.), Type 2 Diabetes and its complications (A preventive program. Microlabe limited: Bangalore, 2008, 1-2.
5. Gupta R, Misra A. Type 2 diabetes in India: regional disparities. British Journal of Diabetes and Vascular Disease 2007; 7:12-16.
6. Abate N, Chandalia M. Ethnicity, type 2 diabetes & migrant Asian Indians. Indian Journal of Medical Research 2007; 125:251-258.
7. Hamdan, Afifi FU. Studies on the *in vitro* and *in vivo* hypoglycemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine. Journal of Ethnopharmacology 2004; 93:117-121.
8. Thalapaneni NR, Chidambaram KA, Ellappan T, Sabapati ML, Mandal SC. Inhibition of carbohydrate digestive enzymes by Talinum portulacifolium (Forssk) leaf extract. Journal of Complementary and Integrative Medicine 2008; 5(1):1-10.
9. Heidari R, Zareae S, Heidarizadeh M. Extraction, Purification, and Inhibitory Effect of Alpha-Amylase Inhibitor from Wheat (*Triticum aestivum* Var. Zarrin). Pakistan Journal of Nutrition 2005; 4(2):101-105.
10. Krishnaveni SB. Theymoli, Sadasivam S. Phenol Sulphuric Acid method. Food Chem 1984; 15:229.
11. Shai LJ, Masoko P, PMokgotho M, Magano S, Mogale MA, Boaduo N *et al.* Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. South African J Bot 2010; 76:465-470.
12. Rajiv GG, Sasikumar P. Antidiabetic effect of *Merremia emarginata* Burm. F. In Streptozotocin induced diabetic rats. Asian. Paci J Tropi Biomedicine 2012; 2:281-286.
13. Rhabaso LR, Chiasson JL. Glucosidase inhibitors. In: Defronzo RA, Ferrannini E, Keen H, Zimmet P. (Eds.), International Textbook of Diabetes Mellitus, Vol. 1, Edn 3, John Wiley and Sons Ltd., UK, 2004, 901-914.
14. Davis SN, Granner DK. Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas. In: Brunton

LL, Lazo JS, Parker KL (Ed.), Goodman and Gilman's: The pharmacological basis of therapeutics, Edn 11, (McGraw-Hill Medical Publication Division: New York, 2001, 1706-1707.
15. Conforti F, Statti G, Loizzo MR, Sacchetti G, Poli F, Menichini F. Traditional Medicinal Herbs and Food Plants Have the Potential to Inhibit Key Carbohydrate Hydrolyzing Enzymes *In vitro* and Reduce Postprandial Blood Glucose Peaks *In vivo*. Biological & Pharmaceutical Bulletin 2005; 28(6):1098-1102.