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ANTIHYPERGLYCEMIC PROTEIN LINEAGE FROM INDIAN MEDICINAL PLANTS

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ABSTRACT

Background: Diabetes is a chronic endocrine disorder caused by lack of insulin and/or reduced insulin activity leads hyperglycemia and abnormalities in carbohydrate, fat-protein metabolism. Numerous medicinal plants have been used for the management of diabetes mellitus in various traditional systems of medicine worldwide as they are a great source of biological constituents and many of them are known to be effective against diabetes due to their antihyperglycemic activities. **Methods:** This current research work was focused on antihyperglycemic proteins profiling from seeds and leaves of aqueous and methanolic extracts of *Momordica charantia*, *Trigonella foenum-graneum*, *Triticum aestivum*, *Solanum tuberosum* and *Nicotiana tobaccum*. Present study was undertaken to analyze the protein profiles from aqueous and methanolic extracts of seeds and leaves of some medicinally important test plants. Crude protein, Osmotin I and II and Polypeptide-P proteins from seeds and leaf. **Result:** The study indicated the presence of antihyperglycemic proteins, thus supporting its traditional medicinal practices. SDS-PAGE revealed that the band of osmotin I at 26KDa. Osmotin I and osmotin II was recovered from seeds and leaves of aqueous and methanolic fraction of wheat, tobacco and potato. The band of Polypeptide-P was obtained at 11KDa from the aqueous and methanolic extracts of seed as well as leaves of bittermelon and fenugreek. **Conclusion:** The methanolic extract of test plants seeds and leaves contained antihyperglycemic proteins except polypeptide-p in aqueous fraction. These proteins act as artificial insulin or analogue for insulin/ animal protein adeponectin or thaumatin. These proteins should be subjected to further purification so as to explore the analysis of the mechanism of action under *in vivo* system *i.e.;* in animal cell lines to observe the bio efficacy of these components in animal model system. Therefore, it may further be serves as an alternative drug of choice.

KEYWORDS: Antihyperglycemic, Protein lineage, Characterization, Osmotin and Polypeptide P.

INTRODUCTION

Diabetes mellitus is one of well known recognized disease of human[1] and on the basis of ancient knowledge about plants and their active metabolites various treatments for the diabetes are explored.[2] Diets of green vegetables, infusions, decoctions, crushed leaves-stems and their aqueous extracts have been used for treatment of diabetes from a long time.[3] Presently, there are around 150 million people suffering from diabetes across global and this will be increased up to 300 million in next decade by 2025. Mainly type 2 Diabetes (T2D) has spread across populations over large geographical areas. Insulin resistance is a major metabolic abnormality of type 2 diabetes. Now a days, researchers show keen interest in identifying insulin-sensitizing agents which counteract insulin resistance in diabetes. Now days, treatment of insulin resistance mainly acts on two mechanisms: peroxisome-proliferator-activating receptors (PPARs) and AMP-activated protein kinase (AMPK). The thiazolidinediones (TZDs) and the biguanides are two agents, used for treatment of insulin resistance. The TZDs are mainly used but associated with some undesirable side effects (weight gain, fluid retention, and heart failure). Another agent, the biguanide metformin does not cause weight gain but acts on liver rather than muscle of human. So, these are not in alone a complete therapy. It is required to search those agents which do not have side effects on human health. Diabetes mellitus is considered leading causes of death in the world [WHO, 2016] which mainly results in higher blood sugar level and also one of the major degenerative diseases. As diabetes has been considered as a clinical model for disease of internal organs.[4] Traditional medicines are best source of inevitable drugs which treat insulin resistance, which provokes researchers in identifying drugs or active

metabolites from plants. The scientific origin for consumption of many of the **"natural medicines"** have not determined till now but a number of active metabolites of different category have been identified[5] as salacinol, an α- glucosidase inhibitor isolated from root of *Salacia reticulate*, identification and successful usage of **metformin**, that was isolated from a plant secondary metabolite, biguanides associated with the legume plant leaves of *Galega officinalis*[6,7], another active molecule as cryptolepine, anindoloquinolene alkaloid extracted from the leaves of *Cryptolepiss anguinolenta.*[8] "Natural medicines", based on plants continues to be used by poor populations globally. It indicates the scientific mechanism for its exploitation is recognized for small number but the medical society does not believe these "natural medicines" as an effective way for the management of thediabetes.[9] Traditional medicines which are used to treat many human diseases since many years, have one of the major advantage is that these does not have side effects and also have vast knowledge about their in vivo efficacy and safety. But in some cases, there are less or no evidences about their in vivo efficacy and mechanism of action. So, it is required to identify compounds and studied about their role in biological system as well as animal models.[10]

Presently based on the existence knowledge it is difficult to say that the "natural medicines" have impact on lowering blood glucose level, with the present proposed project we wish to suggest that in certain conditions the utilization of some plant's active compound it will be possible to add to curative and preventive health care management. Earlier information specify that absorbed insulin along with protease inhibitors is found to be prevented from hydrolytic breakdown in the alimentary canal, pass through the intestinal barrier and supports in lowering of blood glucose level.[11] Furthermore, the separation of galactorhamnan, a polysaccharide along with insulin from Jack bean seed coats indicates the insulin could be sheltered from hydrolysis within the alimentary canal and induces reduction of sugar in blood after passing intestinal barrier.[12,13,14] Comprehensive research academically and medically oriented invention have recognized that insulin not only role as a hormone responsible for utilization of glucose but also concerned with various life processes.[15] A variety of signaling pathways which involves insulin as one of their component are known but their mechanism is still unknown.[16] Besides its traditional function in metabolic pathways linking glucose and insulin has been found in both invertebrate and vertebrate respectively.[17]

The presence of insulin from plant sources is not appreciated by the scientific group of people. This prototype and reclaim knowledge suggests that insulin is found to be associated with plants. On the basis of the review it was indicated that in leguminous plants a protein is present which has equivalent amino acid series like bovine insulin. Furthermore, evidences are observed that proteins which are involved in insulin signalling pathways of vertebrates, also detected along with insulin-like molecules of plant origin.[18] On the other hand, current and strong evidence proposes that the occurrence of insulin in plants, indicates that the hormone plays an analogous role in plants as metazoans.[19,20,21]

In this study we aim to gather information on the occurrence of insulin from plant source and outcome of research suggested the incidence of plant insulin, which is currently executed in some laboratories across the globe. During our work, we have adopted a targeted strategy to scrutinize the potent chemical moiety in *Momordica charantia* L. (Cucurbitaceae). This plant is extensively cultivated as vegetable and herb with medicinal properties in various Asian countries and exhibit hypoglycemic effects both in animal and human models.[22] While major chemical constituent of *M. charantia* contain a cucurbitane tri-terpenoids[23], the specific active compound accountable for the antidiabetic potential of this plant had not been recognized. In this study, we intend to investigate the actual chemical constituents of bittermelon which possess antidiabetic potential.

Polypeptide-p is insulin- like hypoglycemic protein, responsible for reducing blood glucose levels in gerbils, langurs and humans when it is injected subcutaneously. It works in a similar manner as human insulin in body. So, it may be utilized as a plant-based insulin replacement in patients suffering from type-1 diabetes. The ingested extract of bitter melon seeds does produce hypoglycemic effects in type-1 diabetic rats. This reveals that apart from p-insulin, a number of compounds also found in bitter melon seeds which are effective in the treatment of type 1 diabetes.[4,24,25] *Trigonella foenum-graecum* (fenugreek) seeds have also hypoglycemic effect which is demonstrated in cell culture, animal models and human studies.[26]

Bittermelon also contains Polypeptide-P (insulin like polypeptide), which reduces blood sugar level when it is injected subcutaneously into human with type 1 diabetes. Apart from Polypeptide-p, other active metabolites such as charantin, vicine, glycosides, and karavilosides also improve blood sugar levels by enhancing glucose uptake and glycogen synthesis in the liver, muscles, and adipose cells.[27]

Several studies have shown hypoglycemic activity of fenugreek seeds in both animal and human studies, thus, support its established use.[3]

Further, intensive research suggested that fenugreek is responsible for reducing plasma cholesterol level and also triglyceride levels. High content of soluble fiber is responsible for the hypoglycemic effect of fenugreek, its mode of action is known which suggests that it works by decreasing the rate of gastric emptying thereby delay glucose absorption from small intestine. A major active

metabolite present in fenugreek is hydroxyl-isoleucine, which represents 80% of the total free amino acids in fenugreek seeds, also possess insulin-stimulating properties. Fenugreek also contains other compounds such as trigonelline and coumarin having hypoglycemic properties. Bitter Gourd (Momordica charantia) has been widely used as traditional medicine for diabetes. But its mode of action is still unknown, preliminary evidence reveals that it may help to stimulate release of insulin or glycogen synthesis in liver.

Furthermore, bittermelon contains a wide variety of antidiabetic compounds. Although Polypeptide-P has been isolated from fruit of *M. charantia* and possess hypoglycemic activity when injected subcutaneously into patient with type I diabetes.

Unripe fruit of *Momordica charantia* also have role in treatment of type 2 diabetes. It has the capability to reduce insulin which suggests its preventive role in hyper insulinemia condition.[28] Various studies reveal its use in diabetes as well as cancer patients.[22]

A critical strategy for prevention and management of type 2 diabetes is treatment of insulin resistance. Many studies reported that crude extracts from each and every part of *Momordica charantia* L. is the effective treatment of diabetes. Although, the active metabolites which are responsible for the hypoglycemic effect and their actions have not been well characterized due to absence of an appropriate assay and screening system. In order to find hypoglycemic components from bittermelon various cell-based, non radioactive and non fluorescent screening methods were demonstrated. It was revealed that triterpenoids, potent hypoglycemic component of plant and its action involves AMP-activated protein kinase. Compounds which overcome insulin resistance and activate AMP-Activated Protein Kinase.were identified from the Stem of *Momordica charantia* by using cell-Based screening.

Osmotin, a multifunctional plant protein confers resistance to both biotic and abiotic stresses. Level of Adiponectin, an anti diabetic and anti atherosclerotic protein is lowered in obese patients and leads to various diseases such as coronary artery disease, liver diseases and inflammation. Osmotin, a plant protein shows similarity with human hormone adiponectin and it not only induces activity of AMP kinase in mammalian myocytes via. AdipoRs but also binds to the AdipoR1 by activating the similar signaling pathway of adiponectin.[29] Osmotin is recommended as a versatile plant protein for treat almost any disease prevalent in man.[29] Antihyperglycemic proteins are those proteins which lowers blood glucose level i.e. counteracting the high blood glucose level. A plant analogue of adeponectin as antihyperglycemic proteins (osmotin I & II) are isolated from five test plants as Wheat[30,31], Potato[32,23] and Tobacco.[33,34,35] These test plants were chosen for our study as they contain antihyperglycemic proteins. Osmotin I and II are present in wheat, potato and tobacco where as Polypeptide-p are present in bittermelon.[36,37,38]

The concept of plant as a food as well as medicine has a central attraction in diabetic and nutritional sciences. These plants have been used as nutritional supplements as well as medicines across world for diminishing symptoms and conditions in diabetes. Bittermelon, fenugreek, wheat, and potato have been widely studied for its medicinal properties to treat various diseases. The present study aims to isolate, quantify, and characterize the proteins responsible for exhibiting antihyperglycemic activity in five test plants as Bittermelon, fenugreek for polypeptide P; wheat, potato, tobacco for osmotin I and osmotin II. The plant samples subjected for the analysis were seeds and leaves in both aqueous and methanolic fractions. Comparative analysis of the yield of the protein in the plants employed with both the solvents were established. Since these test plants were reported to possess antihyperglycemic activity that gives us impetus to conduct the protein lineage study. So the present need is to explore the component acting as a source of artificial insulin or sensitizer for insulin.

MATERIALS AND METHODS

Test plants

Five plants were used for the analysis of their antihyperglycemic activity in their seeds and leaves as bittermelon (*Momordica charantia*), fenugreek (*Trigonella foenum-graecum*), wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), and tobacco (*Nicotiana tobaccum*). Seeds were collected from local market. These seeds were sown in the earthen pots and provide optimal nutrients, adequate amount of light and water for growth of healthy plants than the leaves were harvested and used for the study conduct. Seeds which were collected from local market and plant extract were prepared by soaking 2 grams of grinded seeds in 0.5 ml in distilled water (aqueous extract) and in methanol (methanolic extract) for overnight. The grinded seeds were subjected to aqueous as well as methanolic extraction for the isolation and quantitation of various protein fractions respectively. Similarly extracts from the leaves were also prepared for the study conduct.

Isolation of osmotin I and II

Samples were crushed in extraction buffer I [20M Pot. Phosphate Buffer (pH 6.5), 5mM EDTA, 1mM PMSF (100mg/ml)] homogenized using vortex mixer. The sample vials were centrifuge at 10,000rpm for 10mins. The supernatant was collected in a separate vial and label as Fraction I which was aqueous soluble. The pellet was resuspended in extraction buffer I and recentrifuge. Pool supernatants obtained in the previous steps in the vial labeled as Fraction I. The pellet was resuspended in extraction Buffer II [20mM Pot. Phosphate buffer, 2mM PMSF, 4M urea, 0.2% NP40 or Triton 100X] and homogenized. The vials were centrifuge at 10,000rpm for 10mins. The supernatant was collected separately and

labeled as Fraction II which is detergent soluble. Protein precipitation/purification using ammonium sulphate salt fractionation: Fraction I which is aqueous soluble was purified using 40% and 80% ammonium sulphate salt solution simultaneously and resuspended the pellet in 100 µl phosphate buffer solution. Fraction II which is detergent soluble was purified using 80% ammonium sulphate salt solution and resuspended the pellet in 100 µl phosphate buffer solution.[39]

Isolation of Polypeptide – p

Leaf, fruit, seed, seed coat suspension culture and callus (100g each) were frozen. The frozen samples were crushed in 10ml of distilled water 45ml of 95% ethanol and 3.6ml of H2SO4 was added and stirred vigorously for 15-20 mins. at 25-28°C, homogenized by adding 60ml distilled water. Than 20 ml of 95% ethanol was added separately, filtered and the pH was adjusted to 3.0 using Ammonium hydroxide (28%v/v). To the flask 1.5 L of acetone was added and kept at 5°C for 8-10hr.[24,25,40]

Quantitation and Characterization of protein

The protein fractions (mg g1-dp or defatted powder) assay according to Folin-Lowry method.[41] SDS PAGE of protein sample was performed.[42] SDS-PAGE was performed using 10% resolving and 5% stacking gels. After electrophoresis at 75 V, protein bands were stained using commesie brilliant blue G 250 and finally the relative density of protein bands were analyzed by Image.

RESULT AND DISCUSSION

The seeds were sown 5 seeds per pot for recovering leaves for the study conduct. Osmotin I and osmotin II and polypeptide-P were isolated, re-suspended in phosphate buffer and were subjected to quantification by Lowry's method and further characterization was done by SDS-PAGE. The present study aimed at isolation and characterization of antihyperglycemic components from bittermelon, fenugreek, wheat, potato and tobacco. The primary aim is to isolate and characterize the active ingredients responsible for antihyperglycemic properties as osmotin I and II (ubiquitous protein – a plant analogue of adeponectin) and polypeptide-P (artificial insulin). Similar report is given by the study on bittermelon that its fruit is utilized for treatment of diabetes and related conditions among the population of various countries including India, also its hypoglycemic effects is known through various postulated mechanisms.[36,37,38] Another study emphasis on highlighting antidiabetic activity as well as phytochemical profiling of *M. charantia* which makes it suitable to explore its therapeutic effects on diabetes[4]. It has been reported that soaked seeds of fenugreek in hot water acts as accessory in treatment of type 2. Whereas an effort has been made to investigate clinically the antidiabetic activity of FG (*Trigonella foenum-graceum Linn.*) and Bael leaves (BL) (*Aegle marmelos*, Corr.) individually and collectively in NIDDM patients by. It has also been reported that a significant hypoglycemic activity shown by fenugreek seeds in T2D patients and its aqueous extract induces insulin signalling pathway.[29] Another study by[40] revealed that novel Fenugreek preparation correcting metabolic amendments associated with diabetes by revealing insulin-like properties and has a probable for clinical implications.

During the study **crude protein** was isolated in aqueous and methanolic fraction from test plants (seeds and leaves). From **seeds** maximum amount of crude protein was recovered from potato aqueous fraction and bittermelon methanolic fraction respectively. During the separation of the crude protein extract from seeds seven bands were recovered from wheat (A) followed by 5 bands in Tobacco (both extracts), bittermelon (M); 4 bands in bittermelon; 3 bands in potato (methanolic), 2 bands in fenugreek, 1 band in wheat (M) and no band in potato (A). The protein band was commonly found in all the extract was 55KDa, 36KDa and 26KDa in all the samples. From **leaves** maximum amount of crude protein was recovered from bittermelon aqueous fraction and fenugreek methanolic fraction respectively. During the separation of the crude protein extract from leaves seven bands were recovered from potato (A) followed by 5 bands in fenugreek (both extracts), bittermelon (M), tobacco (A), 4 bands in bittermelon (A), potato (M), 3 bands in wheat (M), 2 bands in wheat (A) and 1 band in tobacco (both the extracts). The protein band commonly found in all the extract was 55KDa, 36KDa and 24KDa.

Secondly, **osmotin I and II** were isolated in aqueous and methanolic fraction from test plants (seeds and leaves). From seeds highest amount of osmotin I was recovered from tobacco (15.78 mg/gm of tissue), followed by wheat (14.22 mg/gm of tissue), potato (10.0 mg/gm of tissue) in aqueous fraction and tobacco (16.22 mg/gm of tissue) followed by wheat (14.27 mg/gm of tissue), potato (14.17 mg/gm of tissue) in methanolic fraction respectively. Maximum amount of osmotin II was obtained from wheat (19.16 mg/gm of tissue), followed by potato (9.91 mg/gm of tissue), bittermelon (9.73 mg/gm of tissue) in aqueous fraction and wheat (28.4 mg/gm of tissue), followed by tobacco (13.02 mg/gm of tissue), potato (11.87 mg/gm of tissue) in methanolic fraction respectively. Utmost quantity of polypeptide-P was recovered from fenugreek (0.361 mg/gm of tissue), followed by bittermelon (0.238 mg/gm of tissue), wheat (0.214 mg/gm of tissue) in aqueous fraction and fenugreek (-0.112 mg/gm of tissue), followed by bittermelon (-0.120 mg/gm of tissue), tobacco (-0.161 mg/gm of tissue) in methanolic fraction respectively. It indicated that polypeptide P was absent in the methanolic fraction of all the test plants (Table 1).

From leaves highest amount of osmotin I was recovered from potato (19.29 mg/gm of tissue), followed by tobacco (6.13 mg/gm of extract), bittermelon (0.67 mg/gm of tissue) in aqueous fraction and wheat (23.60 mg/gm of tissue) followed by potato (6.22 mg/gmof

tissue), bittermelon (6.09 mg/gmof tissue) in methanolic fraction respectively. Maximum amount of osmotin II was recovered from potato (8.21 mg/gm of tissue), followed by wheat (8.00 mg/gm of tissue), fenugreek (7.6 mg/gmof tissue) in aqueous fraction and wheat (11.11 mg/gmof tissue) followed by fenugreek (9.02 mg/gm of tissue), bittermelon (3.50 mg/gm of tissue) in methanolic fraction respectively (Table 1).

Separation of protein fraction by SDS-PAGE with respect to protein marker (Merck; 29 KDa to 205 KDa). Osmotin I and II, polypeptide P were separated on SDS-PAGE from seeds as well as leaves in both aqueous and methanolic extracts respectively (Figure 1-5). The protein band commonly found in all the extract was 26 KDa and it was not recovered from bittermelon (aqueous and methanolic) whereas during separation of osmotin II bands were recovered from tobacco (aqueous) The protein band commonly found in the extract was 26KDa and it was not recovered from tobacco (aqueous fraction), fenugreek (aqueous and methanolic fraction), bittermelon (aqueous and methanolic fraction), wheat (methanolic and aqueous fraction) and potato (aqueous and methanolic fraction) (Figure 1). From **leaves** maximum amount of osmotin I was recovered from potato in aqueous fraction and wheat in methanolic fraction respectively whereas maximum amount of osmotin II was recovered from potato in aqueous fraction and wheat in methanolic fraction respectively. As during the separation of osmotin I and II from leaves bands were recovered from bittermelon (aqueous and methanolic) I, tobacco (aqueous) I, fenugreek (aqueous and methanolic) II, potato (aqueous and methanolic) I, potato (aqueous and methanolic) II, wheat (aqueous and methanolic) II, wheat (methanolic) I whereas no band was visible in bittermelon (aqueous) II. The protein band commonly found in all the extract was 26 KDa (Figure 2). Similarly a study by [39] revealed that in cultured tobacco cells with osmotin-I and osmotin-II having N-terminal amino acid sequences of both osmotin I and osmotin II are identical till 22 position. Osmotin exhibit similarity to thaumatin in perspective of molecular weight, amino acid sequence, its N-terminal sequence, and presence of signal peptide in precursor protein. Absence of reactivity of thaumatin with anti osmotin. An osmotin has not much sweetness as thaumatin.[23] reported that osmotin proteins are encoded by members of a multigene family in potato. cDNAs encodes for osmotin proteinhave been characterized in potato cell cultures.[23,43] It has been demonstrated that transgenic potato and tobacco has osmotin gene under the control of the CaMV35S promoter which results in constitutively overexpression of osmotin protein to a level of approximately 2% of total cellular protein in plants.[32,44]

Finally, **polypeptide-P** was isolated in aqueous and methanolic fraction from test plants (seeds and leaves). Maximum amount of polypeptide-P was recovered from wheat (0.278 mg/gm of tissue), followed by potato (0.210 mg/gm of tissue), fenugreek (0.087 mg/gm of tissue) in aqueous fraction and wheat (0.622 mg/gm of tissue), followed by fenugreek (0.277 mg/gm of tissue), potato (0.180 mg/gm of tissue) in methanolic fraction respectively (Table 1). On the other hand characteristic band of 11 KDa protein of polypeptide was evident in bittermelon and fenugreek respectively (Figure 4-5). From **seeds** maximum amount of polypeptide-P was recovered from fenugreek in aqueous and methanolic fraction respectively. During the separation of polypeptide-P from seeds bands were recovered from bittermelon (aqueous and methanolic), wheat (aqueous) and tobacco (aqueous fraction). The protein band commonly found in all the extract was 11KDa and it was not recovered from fenugreek (aqueous and methanolic), wheat (methanolic), potato (aqueous and methanolic) and tobacco (methanolic). From leaves, maximum amount of polypeptide-P was recovered from wheat in aqueous and methanolic fraction. During the separation of polypeptide-P from leaves bands were recovered from bittermelon (aqueous and methanolic fraction), fenugreek (aqueous and methanolic), wheat (aqueous and methanolic), potato (aqueous and methanolic), tobacco (aqueous) and bittermelon (methanolic). The protein band commonly found in all the extract was 11KDa (Figure 4-5).[24,25] reported that Polypeptide-p has been extracted from various parts (fruit, seeds) of *Momordica charantia* Linn (bitter gourd). Its amino acid analysis reveals that polypeptide-p has molecular weight of approximately 11 KDa). It is one of the effective hypoglycemic agent when injected subcutaneously to various subjects as gerbils, langurs, and humans[36,37,38]

Table 1: Quantitative analysis of isolated proteins from different test plant (µg/mg)

Protein Fraction	Plant Part	Bittermelon		Wheat		Potato		Tobacco	
		A	M	A	M	A	M	A	M
Crude Protein	Seeds	7.2	12.89	5.51	9.11	8.53	12.22	5.07	8.98
	Leaves	6.27	6.4	0.8	7.24	4.53	15.29	0.755	2.58
Osmotin I	Seeds	9.38	11.73	14.22	14.27	10	14.17	15.78	16.22
	Leaves	0.67	6.09	0	23.6	19.29	6.22	0.159	0
Osmotin II	Seeds	9.73	11.16	19.16	28.4	9.91	11.87	9.11	13.02
	Leaves	3.56	0	8	11.11	8.27	23.51	0	0
Polypeptide P	Seeds	0.238	0	0.214	0	0	0	0	0
	Leaves	0.02	0.097	0.278	0.622	0.21	0.18	0.067	0

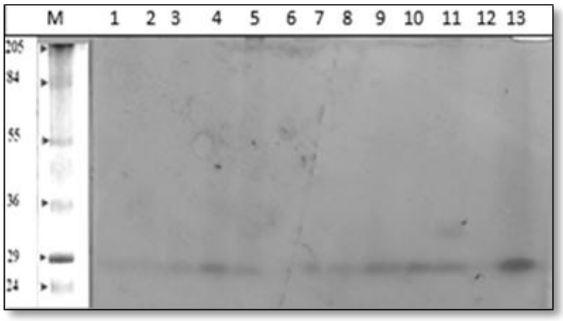

Figure 1: Protein Profiling of the osmotin I in aqueous and methanolic fraction from seeds of the test plants through SDS-PAGE (4% stacking gel and 10% resolving gel): M- Marker (Merck 20.1-205KDa, lane 1: wheat (M)II, 2: tobacco (A) I , 3: potato (A) I, 4: wheat (A) I, 5: fenugreek (A) I, 6: bittermelon (A) I, 7: blank well 8: tobacco (M) I, 9: potato (M) I, 10: wheat (M) I, 11: fenugreek (M) I, 12: bittermelon (M) I, 13: tobacco (M) 1 .

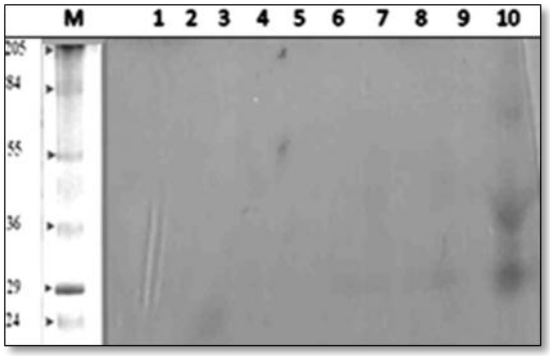

Figure 2: Protein Profiling of the osmotin II in aqueous and methanolic fraction from seeds of the test plants through SDS-PAGE (4% stacking gel and 10% resolving gel): M- Marker (Merck 20.1-205KDa, Lane 1: Bittermelon (M), 2: Bittermelon (A), 3: Fenugreek (M), 4: Fenugreek (A), 5: Wheat (M), 6: Wheat (A), 7:Potato (M), 8: Potato (A), 9: Tobacco (A), 10: Tobacco (M).

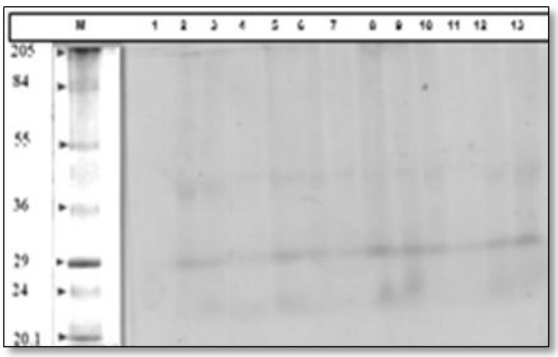

Figure 3: Protein Profiling of the osmotin I and II in aqueous and methanolic fraction from leaves of the test plants through SDS-PAGE (4% stacking gel and 10% resolving gel): M- Marker (Merck 20.1-205KDa, Lane wise 1: bittermelon (A), 2:tobacco (A), 3: fenugreek (A), 4: potato (M), 5:bittermelon (M), 6:potato (A), 7:potato (M), 8: bittermelon (A), 9:fenugreek (M), 10: wheat (A), 11: wheat (M), 12: potato (A), 13: wheat (M).

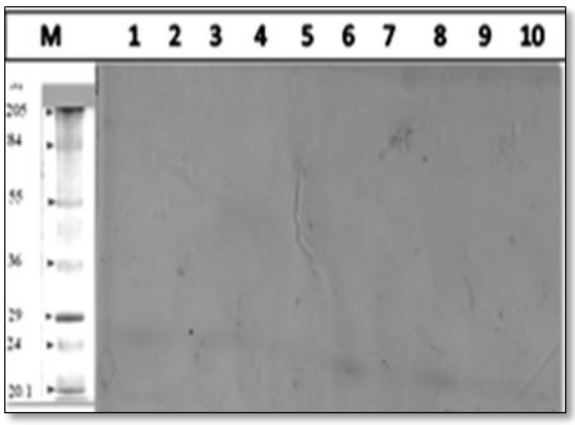

Figure 4: Protein Profiling of the polypeptide- P in aqueous and methanolic fraction from seeds of the test plants through SDS-PAGE (4% stacking gel and 10% resolving gel): M- Marker (Merck 20.1-205KDa, Lane wise: 1:Bittermelon (M), 2: Fenugreek (M), 3: Wheat (M), 4: Potato (M), 5: Tobacco (M), 6: Bittermelon (A), 7: Fenugreek (A), 8: Wheat (A), 9: Potato (A), 10: Tobacco (A).

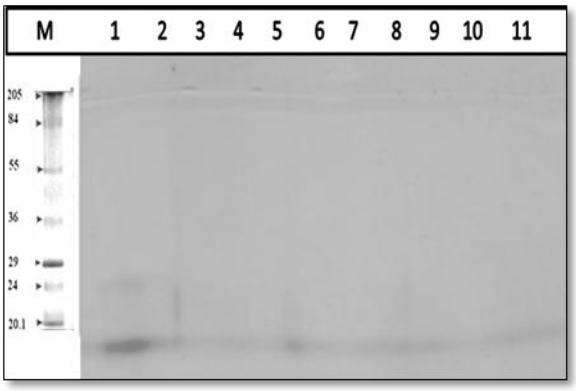

Figure 5: Protein Profiling of the polypeptide- P in aqueous and methanolic fraction from leaves of the test plants through SDS-PAGE (4% stacking gel and 10% resolving gel): M- Marker (Merck 20.1-205KDa Lane wise 1: bittermelon (A), 2: blank 3: fenugreek (A), 4: wheat (A), 5: potato (A), 6: blank 7: tobacco (A), 8: bittermelon (M), 9: fenugreek (M), 10: wheat (M), 11: potato (M).

CONCLUSION

Present study reports the presence, quantitaion and characterization of clear antihyperglycemic protein bands of test plants. As Osmotin exhibit similarity to thaumatin in perspective of molecular weight, amino acid sequence, its N-terminal sequence, and presence of signal peptide in precursor protein Osmotin, ubiquitous plant protein an analogue to adeponectin was commonly found in wheat, potato and tobacco and its protein band was virtually equivalent to 26 KDa and reported in tobacco, wheat, potato and strawberry. Whereas, Polypeptide-P (artificial insulin) was reported from bittermelon and fenugreek

with their protein band equivalent to 11KDa. Theses protein can further be used as curative measures for the treatment of hyperglycemia.

The study can highlight the fact that traditional medicines are effective option for ethnic minorities where a high incidences of diabetes but they prefer natural products for their treatment as per their cultural or traditional beliefs. Relatedness and usefulness of this study is mainly concerned with plants possessing antihyperglycemic property. Since naturopathy plays a significant role in providing a platform to the researchers to pursue scientific interventions in Ayurveda, Naturopathy, indulges in providing a range of integrated treatment which covers various aspects of diseases as protective, therapeutic needs. These systems mainly increasing attention of people across globe. Naturopathy schemes of remedy are being used for centuries and have continuous traditions of recognition and practice.

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Compliance with ethics guidelines

Disclosure of potential conflicts of interest indicates that there is no conflict of interest during the study conduct in any of the authors. The research doesn't involve any animal model studies and human subjects. The study is conducted in compliance with Ethical Standards.

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