



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)

Platform for Pharmaceutical Researches & Innovative Ideas
www.ijprd.com

INVESTIGATION OF *INVITRO* α -AMYLASE AND α -GLUCOSIDASE INHIBITORY ACTIVITY OF POLYHERBAL EXTRACT

Mahesh Bhanudas Narkhede

Department of Pharmacology, IBSS College of Pharmacy, Malkapur (MS)- 443101.

ABSTRACT

One therapeutic approach for treating diabetes (DM) is to decrease postprandial hyperglycemia. This can be achieved by the suppression of carbohydrate hydrolyzing enzymes like α -amylase and α -glucosidase. So inhibitors of such key enzyme offer an effective strategy to lower the levels of post prandial hyperglycemia via control of starch breakdown. Four Ayurvedic Indian medicinal plants with known hypoglycemic properties were subjected for study, in order to assess and evaluate their inhibitory potential. In this research, phytochemical assay and *in vitro* inhibitory assay of α -amylase and α -glucosidase were performed toward polyherbal extracts of *Cynodon dactylon* (Graminae), *Murraya paniculata* (Rutaceae), *Ocimum sanctum* (Labiatae), *Tamarindus indica* (Fabaceae). Our assay results suggests that polyherbal extract (PHE) significantly ($P < 0.05$) inhibits *in vitro* key enzyme linked in DM in concentration-dependent manner. In α -glucosidase inhibitory activity, the percentage inhibition of PHE (100-1000 $\mu\text{g/ml}$) showed a concentration-dependent increase in percentage inhibition. The percentage inhibition varied from 85.71 ± 0.918 to 30.78 ± 0.4855 for highest concentration to the lowest concentration of $100 \mu\text{g/ml}$ where as in α -amylase inhibitory assay, at a concentration $100 \mu\text{g/ml}$ of PHE showed a percentage inhibition of 61.31 ± 0.3729 for $1000 \mu\text{g/ml}$. Acarbose as a synthetic inhibitor of α -glucosidase and α -amylase, was used as a standard drug.

Correspondence to Author

Mahesh Bhanudas Narkhede

IBSS College of Pharmacy,
Malkapur Buldana Road,
Malkapur Dist. Buldana-443 101

Email

maheshnark@gmail.com

Key Words

polyherbal extract; α -amylase; α -glucosidase; acarbose

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion^[1]. Calculation of the excess global mortality revealed that DM is likely to be the fifth leading cause of death^[2]. The frequency of this disorder is on the rise globally, is likely to hit 300 million by 2030 with India projected to have the largest number of diabetic cases^[3]. The most critical complications of DM are post-prandial hyper-glycemia (PPHG). Post-prandial phase is associated with macrovascular and microvascular diabetic complications and it is the major independent risk factor for cardiovascular disease^[4].

One therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved by delaying the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes, α -amylase and α -glucosidase in the digestive tract. α -glucosidase inhibitors can retard the liberation of glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycaemia^[5]. α -amylase and α -glucosidase inhibitors are drug-design targets in the development of compounds for the treatment of diabetes, obesity and hyperlipaemia^[6]. α -Glucosidase inhibitor and α -amylase inhibitors are class of compounds that help in managing PPHG. Low-cost herbal treatment is recommended due to their lesser side effect for treatment of diabetes^[7]. Moreover these days great attention has given to the management of diabetes with medicinal plants. In Ayurveda and other Indian traditional medicine have since ancient times used plants in treatment of diabetes^[8]. Ethnobotanical studies of traditional herbal remedies used for diabetes have identified more than 1,200 species of plants with hypoglycemic activity^[9, 10]. The search for new pharmacologically active agents obtained by screening of medicinal plants or their extracts can lead to potent and specific inhibitors for key enzymes linked in diabetes.

Four Ayurvedic Indian medicinal plants with known hypoglycemic properties were subjected for

study, in order to assess and evaluate their inhibitory potential. *Cynodon dactylon* (Family: Graminae) is folk remedy for skin wound, calculus, cancer, carbuncles, cough, hypertension, stones, gout and rheumatic affections^[11]. Ethanol extract of aerial parts also marked CNS depressant^[12], hypoglycemic^[13], hypolipidemic and antioxidant activities^[14]. *Murraya paniculata* (Family: Rutaceae), is distributed throughout China, India, Sri Lanka and Taiwan. It possesses stimulant and astringent properties^[15]. The plant is known to have emetic, carminative, antipyretic^[16] analgesic^[17] free radical scavenging^[18] and antidiabetic^[19]. *Ocimum sanctum* (Family: Labiateae), is commonly known as Vishnu-Priya, Tulsi in Sanskrit, Kala- Tulsi in Hindi and India's Holy Basil in English. The leaves, seeds and root of this plant have been used in indigenous Ayurvedic medicine. It has been evaluated pharmacologically for antimicrobial, immunomodulatory, anti-stress, anti-inflammatory, antipyretic, anti-asthmatic, hypoglycemic, hypotensive and analgesic activities. Tulsi is one of such drugs, used in diabetes mellitus^[20]. *Tamarindus indica* (Family: Fabaceae) was used as a traditional medicine for the management of diabetes mellitus in human and experimental animals^[21]. The potential antioxidant activity of Tamarind seeds have already been reported^[22].

In this study, we have selected medicinal plants from the Indian Ayurvedic system with known hypoglycemic or antidiabetic activity, in an attempt to screen for α -amylase and α -glucosidase inhibitors. Extract mixture of leaves of *C. dactylon*, *M. paniculata*, and *O. sanctum*, *T. indica*, were selected for *in vitro* antidiabetic activity.

MATERIALS AND METHODS

Drug and Chemicals

Potato starch were purchased from SD Fine Pvt. Ltd., Mumbai, 3,5-dinitrosalicylic acid, Tris buffer from Hi-Media Pvt. Ltd., Mumbai, α -amylase and α -glucosidase enzymes were purchased from SRL Pvt. Ltd., Mumbai. Glucose assay kit from Diagnostic Pvt. Ltd., Mumbai, Acarbose free sample was obtained from Bicon Pvt. Ltd., Chennai.

Plant Materials

Study plant material, *C. dactylon*, *T. indica* leaves were collected from Balaghat region of Buldhana district (Maharashtra) and *M. paniculata*, *O. sanctum* leaves from college campus. All the plants were checked for purity and authenticity by HOD, Nagarjuna Udyan of Dr. PKV, Akola and voucher specimens were deposited in Department of Pharmacognosy, IBSS College of Pharmacy, Malkapur.

Preparation of water Extract

Water extraction was prepared as follows:

The leaves of *Cynodon dactylon*, *Murraya paniculata*, *Ocimum sanctum* and *Tamarindus indica* were shade dried and made into coarse powder in the grinding mill. Mixed powder materials were extracted using water. Dried Powder (75g) of leaves were extracted continuously by masseration using water (200 ml) which then evaporated at 45 ± 5 °C to yield dry water extraction. The extract was preserved in refrigerator till further use.

Phytochemical Assay

Phytochemical assay of all extracts were performed to determine chemical compound content of extracts qualitatively. Based on an established procedure, was conducted to explore the secondary metabolites, such as: alkaloid, flavonoid, saponin, triterpenoid, steroid, and tannin^[23].

INHIBITORY ASSAY OF KEY ENZYMES LINKED IN DIABETES

Inhibitory assay of alpha amylase enzyme

The α -amylase inhibition assay was performed using the chromogenic method. Soluble starch solution was prepared by using potato starch in 25ml of 0.20mM phosphate buffer (pH 6.9). α -amylase (0.5mg/ml) solution was prepared in the same buffer. The colorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3, 5-dinitrosalicylic acid. PHE and standard drug acarbose was dissolved in DMSO^[24].

Test and standard drug of varied concentration in 500 μ l were added to 500 μ l of buffer containing α -amylase solution and were incubated. After these, 500 μ l

of a starch solution was added to each tube. The reaction mixtures were then incubated. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid. The test tubes were then incubated in a boiling water bath and cooled. 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^[25].

Inhibitory assay of alpha glucosidases enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1ml 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 1ml of α -glucosidase enzyme (1U/ml) to it followed by incubation. Then, the reaction mixture was heated in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method^[26].

Calculation of 50% Inhibitory Concentration (IC₅₀)

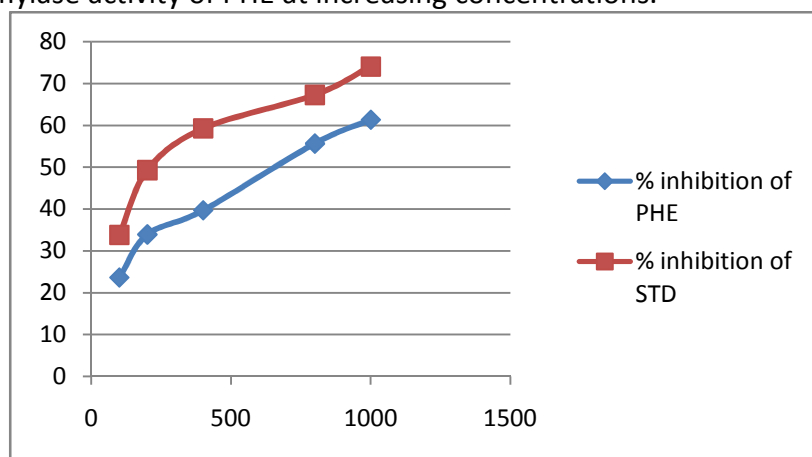
The concentration of the plant extracts required to inhibit 50% of the enzyme (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by $I \% = (Ac - As) / Ac \times 100$, where Ac is the absorbance of the control and As is the absorbance of the sample.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the Polyherbal extract (PHE) of *C. dactylon*, *M. paniculata*, *O. sanctum* and *T. indica* leaves mixture, revealed the presence of flavonoids, alkaloid, saponins, tannins, steroids and triterpenoids. Previous studies showed that, extract of *C. dactylon* present flavonoids and steroids^[27, 28]. *M. paniculata* has reported the isolation of alkaloids, flavonoid and tannin^[29]. Two flavonoids, viz., orientin and vicenin from aqueous leaf extract of *O. sanctum* have been isolated^[30] and also contain a number of sesquiterpenes and monoterpenes^[31]. *T. indica* showed the presence of carbohydrates, flavonoids, pectin, tartaric acid and dihydrobutanedioic acid and tannins^[32].

Recent advances in understanding the activity of intestinal enzymes (α -amylase and α -glucosidase both are important in carbohydrate digestion and glucose absorption) have lead to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with complications in diabetes and is more strongly associated with the risk for cardiovascular diseases than are fasting blood glucose. α -Glucosidase enzymes in the intestinal lumen and in the brush border membrane play main roles in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation [33]. Alpha-glucosidase inhibitor retards the digestion of carbohydrates and slows down the absorption. Acarbose and miglitol are competitive inhibitor of α -glucosidases and reduces absorption of starch and disaccharides [34]. Hence one of the therapeutic approaches for reducing postprandial (PP) blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition of these enzymes (α -amylase and α -glucosidases) reduced the high postprandial (PP) blood glucose peaks in diabetes [35]. Acarbose and miglitol are competitive inhibitor of α glucosidases and reduces

Fig 1. : The inhibition of α -amylase activity of PHE at increasing concentrations.



absorption of starch and disaccharides [34]. The α -amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. Synthetic inhibitor causes side effect such as abdominal pain, diarrhoea and soft faeces in the colon. So, in order to overcome, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. Based on the traditional use of the plant as anti-diabetic and anti-hyperlipidemic effect, the present study was carried out *in vitro* antidiabetic study to substantiate the folklore claim.

Our finding reveals that water extract of herbs selected for inhibitory assay, efficiently inhibits α -amylase enzyme *in vitro*. There was a dose-dependent increase in percentage inhibitory activity against α -amylase enzyme by PHE. At a concentration $100\mu\text{g/ml}$ of extract showed a percentage inhibition 23.62 ± 0.2454 and for $1000\mu\text{g/ml}$ it was 61.31 ± 0.3729 (Fig 1). The PHE gave an IC_{50} value of $540.90 \pm 3.04\mu\text{g/ml}$. The IC_{50} value of standard drug acarbose was found to be $330.50 \pm 4.7\mu\text{g/ml}$ (Table 1). The reaction mechanisms involved in inhibition of α -amylase enzymes by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein (flavanols) might cause conformational changes in structure [36].

All determinations were carried out in triplicate manner and values are expressed as the mean \pm SEM. Acarbose was used as a positive control.

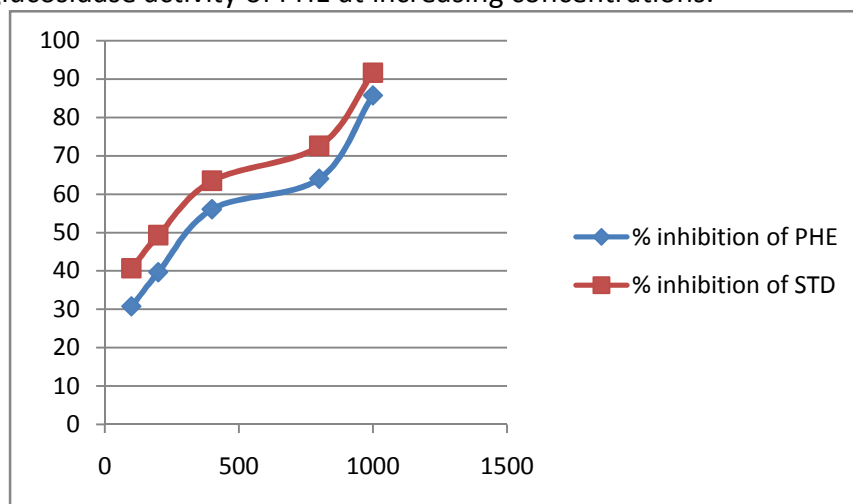
Table 1: Effects of polyherbal water extracts (PHE) concentrations on % reduction α -amylase and α -glucosidase enzyme *in vitro*

	IC50 values (mg/ml)	
	α -amylase enzyme	α -glucosidases enzyme
PHE (Water extract)	540.90 \pm 3.04	425.20 \pm 6.08
Acarbose	330.50 \pm 4.7	295 \pm 3.80

The IC₅₀ value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. Acarbose was used as a positive control.

The PHE revealed a significant inhibitory action on α -glucosidase enzyme. The percentage inhibition at 100-1000 μ g/ml concentrations of extract showed a concentration-dependent increase in percentage inhibition. The percentage inhibition varied from 85.71 \pm 0.918 to 30.78 \pm 0.4855 for highest concentration to the lowest concentration of 100 μ g/ml. The IC₅₀ was found to be 425.20 \pm 6.08 μ g/ml whereas α -glucosidase inhibitory activity of positive control acarbose produced percentage of 40.73 \pm 1.39 for 100 μ g/ml and 91.58 \pm

1.39 for 1000 μ g/ml (Fig 2). The IC₅₀ value of standard drug acarbose against α -glucosidase was found to be 295.71 \pm 3.80 μ g/ml (Table 1). The presence of inhibition to alpha-glucosidase activity of water extract of polyherb leaves could be caused by the presence of carbohydrate, plant protein (flavanols) which is suspected to be the competitive inhibitor for alpha-glucosidase enzyme. This is appropriate with the substrate of alpha-glucosidase which is food carbohydrate, such as starch and glycogen.

Fig 2.: The inhibition of α -glucosidase activity of PHE at increasing concentrations.

All determinations were carried out in triplicate manner and values are expressed as the mean \pm SEM. Acarbose was used as a positive control.

CONCLUSION

Polyherbal extract mixture of leaves of *C. dactylon*, *M. paniculata*, *O. sanctum* and *T. indica* is a good source of water-extractible phytochemicals with strong antioxidant activities and inhibitory activities against key enzymes linked to type-2 diabetes, namely, α -amylase and α -glucosidase. Thus offers a potential for use as dietary intervention in the management or control of postprandial hyperglycemia associated with type-2 diabetes.

ACKNOWLEDGEMENT

We are thankful to the Management, Staff of MRD lifeSciences (P) Limited, Lucknow, and specially Mrs. Nilofar Khan for their kind support throughout the research work, we are also thankful to God without whose consent nothing is possible.

REFERENCES

- World Health Organisation Consultation: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Report of a WHO Consultation Geneva; 1999
- Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S *et al.*, The burden of mortality attributable to diabetes: realistic estimates for the year 2000, *Diab. Care* 2005; 28: 2130-2135
- Gupta OP, Phatak S: Pandemic Trends in Prevalence of Diabetes Mellitus and Associated Coronary Heart Disease in India: Their Causes and Prevention. *Int J Diabetes Dev Countries* 2003; 23:37-50
- Bonora E, Muggeo M. Postprandial blood glucose as a risk factor for cardiovascular disease in Type II diabetes: Epidemiological evidence. *Diabetologia* 2001; 44:2107-14
- Lebovitz HE: Alpha-glucosidase inhibitors. *Endocrinology and Metabolism Clinics of North America* 1997; 26: 539–551
- Franco OL, Rigden DJ, Melo FR, Grosside-Sa MF. Plant α -amylase inhibitors and their interaction with insects α -amylases. Structure, function and potential for crop protection. *European Journal of Biochemistry* 2002; 269:397-412
- Ghosh S, Ahire M, Patil S, Jabgunde A, Dusane MB, Joshi BN *et al.*, Antidiabetic Activity of *Gnidia glauca* and *Dioscorea bulbifera*: Potent Amylase and Glucosidase Inhibitors. *Evidence Based Complementary & Alternative Medicine* Vol. 2012, Article ID 929051, 10 pages, 2012
- Babu PA, Suneetha G, Boddepalli R, Lakshmi VV, Rani TS, Rambabu Y, Srinivas K: A database of 389 medicinal plants for diabetes. *Bioinformation* 2006; 1(4): 130-171.
- Leena AA, Jill PC: Type 2 Diabetes Prevention: A Review. *Clinical Diabetes* 2010; 28(2):53-59
- Farnsworth NR: In Screening plants for new medicines. Edited by: Wilson EO. Biodiversity National Academy Press, Washington DC; 1998:83-97
- Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi: Publication and Information Directorate 1999 p.88
- Pal DK. Evaluation of CNS activities of aerial parts of *Cynodon dactylon* Pers in mice. *Acta Pol Pharm-Drug Res* 2008; 65: 37-43
- Singh SK, Kesari AN, Gupta RK, Jaiswal D, Watal G. Assessment of antidiabetic potential of *Cynodon dactylon* extract in streptozotocin diabetic rats. *J Ethnopharmacol* 2007; 114: 174-9
- Pal DK, Kumar M, Chakrabarty P, Kumar S. Evaluation of the antioxidant activity of aerial parts of *Cynodon dactylon*. *Asian J Chem* 2008; 20: 2479-2481
- Parrotta JA. Healing plants of Peninsular India. Wallingford, UK and New York: CABI Publishing 2001; p.917
- Chopra RN, Chopra IC, Hunda KI, Kapoor LD. Chopra's Indigenous Plants of India. New Delhi, India: Academic Publishers 1982; p. 1-5
- Chevallier A. The Encyclopedia of medicinal plants. 1st Ed. New York: DK publishing Inc.;1996
- Vijaykumar M, ChV Rao, Rawat AKS *et al.*, Free radical scavenging and lipid peroxidation inhibition potential of various fraction of *Murraya paniculata*. *Pharmacologia* 2012; 3 (5) : 138-143
- Gautam MK. Effect of *Murraya paniculata* leaves on streptozotocin induced oxidative stress in diabetic

- rats. M Pharm Thesis, Vinayaka Mission's Research Foundation, Deemed University, Salem – 636008; 2007
20. Chiang L C, Ng L T, Cheng P W, Chiang W & Lin C. Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. *Clinical and Experimental Pharmacology and Physiology* 2005, 32(10): 811-816
 21. Maiti R, Jana D, Das UK and Ghosh D. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats. *J. Ethnopharmacol* 2005; 92(1): 85-91
 22. Dighe NS, Pattan SR, Nirmal SA, Kalkotwar RS, Gaware VM and Hole MB Analgesic activity of *Tamarindus indica*. *Research J. Pharmacognosy and Phytochemistry* 2009;1(1): 69-71
 23. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 31st ed. Pune (India): Nirali Prakashan; 2005 p. 593-597
 24. Bernfield P: Enzymes of starch degradation and synthesis. *Advances in Enzymology* 1951; 12. p.379
 25. Thalapaneni NR, Chidambaram KA, Ellappan T, Sabapati ML, Mandal SC. Inhibition of carbohydrate digestive enzyme by *Talinum portulacifolium* (Forssk) leaf extract. *Journal of Complementary and Integrative Medicine* 2008; 5(1): 1-10
 26. Andrade-Cetto A, Becerra-Jimenez J, Cardenas-Vazquez R. α -glucosidase inhibitory activity of some Mexican plants used in the treatment of type 2 diabetes. *Journal of Ethnopharmacology* 2008; 116: 27-32
 27. Aishah, HS, Amri AMM, Ramlan MF, Mamat AS. Organic materials and nitrogen-potassium ratios for Bermuds tifdwarf (*Cynodon dactylon*). *Acta Horticulture* 1997; 450: 505-510
 28. Patil MB, Jalalpure SS, Prakash NS, Kokate CK. Antiulcer properties of alcoholic extract of *Cynodon dactylon* in rats. *Acta Horticulture* 2005; 680:115–118
 29. Takeshi K and Kurnia F. Myricetin 5,7,3',4',5'-Pentamethylether and other methylated flavonoids from *M. paniculata*. *Phytochemistry*.1997;45 (1): 179- 181
 30. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* L. as a medicinal plant. *Indian J Exp Biol* 2002; 40: 765-773
 31. IDMA. Indian Herbal Pharmacopoeia. Mumbai, India: 2002, p. 272
 32. Mute VM, Sampat VM and Patel KA *et al.*, Anthelmintic effect of Tamarind Indica L. Leaves juice extract on *Pheretima Posthuma*. *International J of Pharmaceutical Research & Development* 2009; 7(001): 1-7
 33. Puls W, Keup U, Krause HP, Thomas G, Hoffmeister F. *Naturwissenschaften* 1997; 64:536
 34. 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, 11th ed. (McGraw-Hill Medical Publication Division: New York 2001:1706-1707
 35. Conforti F, Statti G, Loizzo MR, Sacchetti G, Poli F, Menichini F. *Biological & Pharmaceutical Bulletin* 2005; 28 (6): 1098-1102
 36. Kim JS, Kwon CS, Son KH. *Bioscience Biotechnology and Biochemistry* 2000; 64(11): 2458-2461
