Study on *in- vitro* antidiabetic potential of stem part of *Musa paradisiaca* Linn

Ajay Singh Bisht[1,*], Divya Juyal[2], Monika[3], Sangeeta Verma[4], Sweeta Joshi[5]

[1,3]Assistant Professor, [2]Director, [4,5]Student, Himalayan Institute of Pharmacy & Research, Dehradun, Uttarakhand

***Corresponding Author:**
Email: ajay86_pharma@yahoo.com

Abstract

The study proposed to evaluate the Phytochemical and *in-vitro* antidiabetic potential of the stem part of *Musa paradisiaca* Linn. *Musa paradisiaca* is commonly known as banana. Its fruit is generally used as dietary source. Various pharmacological activities have been investigated in leaves, fruits, and fruit pulp of this plant. But very few activities and researches have been done on stem part of the plant. Generally stem of the plant considered to be the waste part. So we have used the same to investigate its phytochemical constituents and *in vitro* anti-diabetic potential by using alpha amylase and alpha glucosidase inhibition method. Results found that various phytochemical constituents are present in the hydro-alcoholic and methanolic extracts in different percentages like starch (4.31%, 3.79%), sugar (0.61%, 0.57%), flavanoids (0.45%, 0.35%), tannins (1.33%, 1.29%), phenolic compounds (5.4%, 4.5%), proanthocynidine (7.4%, 7.0%), and flavonols (0.72%, 0.64%) respectively.

Keywords: *Musa paradisiaca,* alpha amylase, alpha glucosidase, Phytochemical, Proanthocynidine.

Introduction

Natural products are used to cure disease and illness with therapeutic properties from ancient time as human civilization and, for a long time. Mineral, plant and animal products were the main sources of drugs.[(1)] *Musa paradisiaca* (linn.), a member of family Musaceae is well known as Plantain or banana. *Musa paradisiaca* is a monoherbacious plant, belonging to family Musaceae, commonly known as plantain. Plantain refers in India to a coarse banana. The plants having two genera and 42 different species, 35 species are belongs to Musa species.[(2)] It is up to 9 m long with a robust tree like pseudostem, large elongated oval deep-green leaves (up to 365 cm in length and 61 cm in width), with a prominent midrib, every plant produces a single inflorescence like drooping spike and large bracts opening in succession, ovate, 15-20 cm long, concave, dark red color and in somewhat fleshy. Fruits are oblong, fleshy, 5-7cm long in wild form and longer in the cultivated varieties.[(3)] The plant is widely distributed in the parts of West and East Africa, Nigeria, Malaysia, Camroon and southern parts of United States.[(4)] Detailed studies in pharmacological investigations revealed that banana fruits, stem juice and flowers are screened for analgesics activity,[(5)] it also shows hair growth promoting activity,[(6)] some articles shows it possess anticonvulsant activity,[(7)] and antimicrobial activity. Shoots - The juices of *Musa balbisiana* have been reported for dissolving pre-formed stones and in preventing the formation of stones in the urinary bladder of rats.[(8)] Flower consists of tannins, saponins, reducing and non-reducing sugars, sterols and triterpenes. The structure of new tetracyclic triterpine isolated from the flowers of *Musa paradisiaca* Linn was determined as (24R)-4α-14α, 24-trimethyl-5-cholesta-8, 25 (27)-dien-3β-ol.[(9)] Banana flowers were investigated as a potential source of natural colorant.[(10)] Fruit consists of carbohydrates, amino acids, sugar and starch. Foremost components of this starch are amylose and amylopectin, present in a ratio of around 1:5. About 1.3% of sugars are present in total dry matter in unripe plantains, but studies shows this raises to around 17% in the ripe. The skin of the fruit is rich in cellulose (10%), hemicelluloses (7%). The pulp protein was rich in arginine, aspartic acid, glutamic acid, methionine and tryptophan.[(11)] Sucrose synthetase is present in the highest concentration in root stock and fruit pulp considerable variations exist in the content of glucose, fructose, sucrose, starch and protein.[(12)] Sucrose phosphate synthetase in the pseudo stem. Acid invertase is present in leaves, leaf sheath and fruit pulp and root stock. The maximum activity of ATP/D-phosphoglucose pyrophosphorylase is found in root stock. Hexokinase is most active in root stock. Acid phosphotase and alkaline phosphatase activity is highest in fruit pulp and pseudo stem. Glucose phosphate isomerase is most active in the root stock and lowest in the leaves.[(13)]

Material and Methods

Plant material: *Musa paradisiaca* stem were collected from Gudrich Vikasnagar, Uttrakhand, India and authentified by Forest Research Institute (FRI), Dehradun Uttrakhand, India.

Preparation of extract: The stem was cut horizontally circular pieces and then dried in shade. And then grind into powdered form and finally sieved to get uniform powdered drug. The powdered plant material (15g) was subjected to maceration using ethanol for 4 days, then filtered with muslin cloth and evaporated to dryness. Extract was kept in desiccator.

Scientific studies: Pharmacognostical and Phytochemical evaluations were carried out from shade dried plant part powder. Estimation of total Sugar and

total starch of plant material was carried out with according to Mont Gomery, 1957 [Spectrophotometric method] taking dextrose and starch (soluble), respectively as a standard solution. Total tannins were determined by using Tannic acid as standard and Gallic acid for the determination of total phenolics. For the determination of total flavanoids and total flavonols Rutin was taken as a standard. Proanthocynidines were estimated by using Catechin as a standard.

Antidiabetic Activity

Inhibition of alpha-amylase enzyme: A starch solution (0.1% w/v) was obtained bystirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3,5-dinitro salicylic acid solution 96 mM. Both control (Acarbose std. drug) and synthesized compound(s) were added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3,5-dinitro salicylic acid to 3- amino- 5- nitro salicylic acid. This reaction is detectable at 540 nm.

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 control (Acarbose std. drug) and the synthesized compound(s) 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 540nm.

Calculation of 50% Inhibitory Concentration (IC 50): The concentration of the synthesized compounds 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 100. Here Ac = absorbance of the control and As = absorbance of the sample.

Results and Discussion

Table 1: Phytochemical screening of successive fraction from soxhlet, (+) shows presence, and (−) shows absence of content of stem

S. No.	Compound	Test	Pet. ether	n-hexane	Chloroform	Ethyl acetate	Methanol	Water
1.	Carbohydrates	Molish' test	-	+	-	+	-	+
		Fehling's test	-	+	-	+	-	+
		Benedict's test	-	-	+	+	-	+
2.	Protein	Biuret tet	+	+	+	+	-	+
		Millon test	-	+	-	+		+
3.	Amino acids	Ninhydrin test	+	+	+	+	-	+
4.	Fats and oils	Solubility test with chloroform	+	+	+	+	+	-
5.	Flavonoids	Alkaline test	-	+	-	-	++	++
		Zinc hydrochloride test	-	-	+	-	++	++
6.	Glycosides (saponin)	General test	-	+	-	+	-	+
		Froth test	-	+	-	+	-	+
7.	Alkaloids	Dragendorff's	+	-	+	+	-	+
		Mayer's	+	-	+	+	-	+
		Wagner's	+0	-	+	+	-	+
		Hager's	+	-	+	+	-	+
		Tannic acid	-	-	+	+	-	+
8.	Phenolic compound (tannins)	Chlorgenic acid	++	+	+	+	++	+

Table 2: Percent of different components in methanol and hydro-alcoholic extract of stem of *Musa paradisiacal*

Content sample	% content Hydro-alcoholic	% content Methanol
Sugar	0.61	0.57
Starch	4.31	3.79
Tannin	1.33	1.29
Phenolic compound	5.4	4.5
Flavanoids	0.45	0.35
Flavonols	0.72	0.64
Proanthocynidine	7.4	7.0

Antidiabetic Activity

***In Vitro* – Alpha Amylase Inhibition Method**

Table 3: Shows % inhibition of alpha-amylase enzyme

	Extracts	% inhibition concentration of sample (µg/ml)				
		0.2	0.4	0.6	0.8	1.0
1	Methanol	20.05	36.18	51.15	71.21	74.62
2	Hydro-alcoholic	28.34	48.16	63.19	82.62	84.53
3	Standard	40.83	68.45	72.66	88.67	96.34

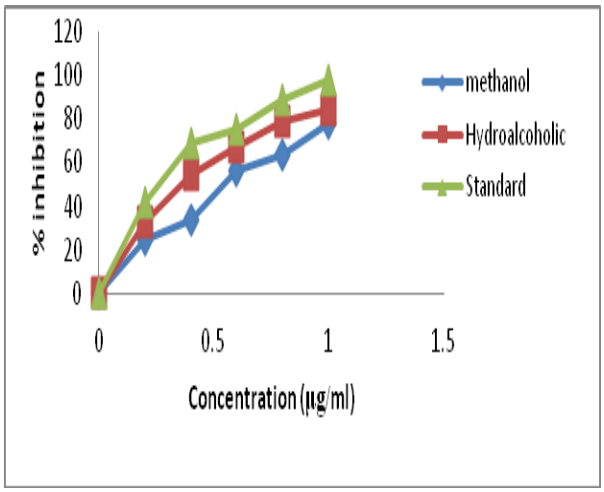

Fig. 1: *In-vitro* alpha-amylase inhibition activity

***In Vitro* Alpha-Glucocidase Inhibition Method**

Table 4: shows % inhibition of alpha glucocidase enzyme

S. No.	Extracts	% Inhibition concentration of sample (ig/ml)				
		0.2	0.4	0.6	0.8	1.0
1	Methanol	24.30	33.43	56.26	63.81	78.17
2	Hydro-alcoholic	32.84	54.43	66.26	78.81	84.17
3	Standard	41.77	69.35	75.33	88.89	97.96

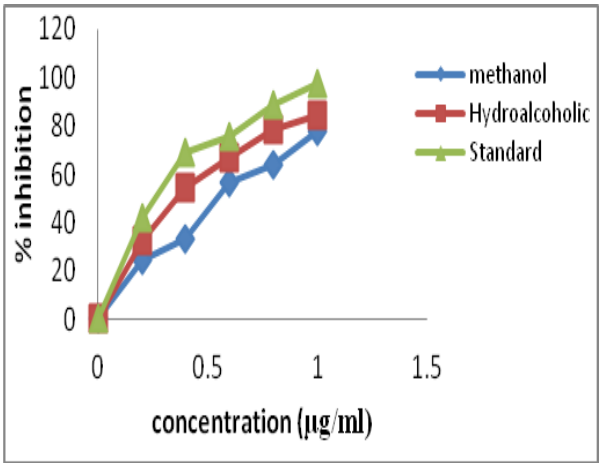

Fig. 2: In vitro alpha-glucosidase inhibition

Conclusion

The present study attempts to investigate the phytochemical estimation and*in-vitro* antidiabetic activity of methanol and hydro-alcoholic extract taken from stem of *Musaparadisiaca.* Resultsrevealed that mostly phenolic and flavanoidal compounds are present in stem part of the plant. The present findings divulge that the extracts efficiently inhibit both alpha amylase and alpha glucosidase enzymes *in vitro* in a dose dependent manner. Data disclose that the extracts have significant inhibitory activity, but comparatively hydroalcoholic extract found to be most active against the enzymes.

References

1. Pasquale D. A., 1984. Pharmacognosy the oldest modern science. *Journal of Ethnopharmacology*, Vol.11, pp. 1–16.
2. Evans W.C. & Trease., 2002. Pharmacognosy. Saunders Elsevier, 16th Edn., Vol. 42.
3. Dutta P.K., Das A.K., Benerji N., 1986. Phytochemistry., Vol. 22 (11), pp. 2563.
4. OlorunfemiA. E., ObotS., JacksonU., AkeemA., 2010. *International Journal of Phytopharmacy research*, Vol. 1, pp. 21-24.
5. Gupta S, Garg VK, Sharma PK, Singh A. 2011, Analgesic activity of aqueous extract of Musa paradisiaca. *Der Pharmacia Sinica* 2 (4):74-77.
6. Savali AS, Bhinge SD, Chitapurkar HR. 2011. Evaluation of hair growth promoting activity of *Musa paradisiaca* unripe fruit extract. *Journal of Natural Pharmaceuticals*, Vol. 3, pp. 120-124.
7. Hallikeri C.S., Suresh H.M., Chandur V.K., Bhoomannavar V.S., Shivakumar S.I., Hatapakki B.C., Alagawadi K.R., 2008. Anticonvulsant effect of the unripe fruits of Musa paradisiaca in albino rats. *Phytopharmacology and therapeutic values*, pp. 433-438.
8. Swathi D., Jyothi B., Sravanthi C., 2011. A Review: Pharmacognostic studies and Pharmacological actions of Musa paradisiaca. *International Journal of Innovative Pharmaceutical Research*, Vol. 2(2), pp. 122-125.
9. Adegboyega O., Ketiku., 2006. *Journal of the science of food and Agriculture*, Vol. 24 (6), pp. 703.

10. Jang D.S., Park E.J., Hawthorne M.E., Vigo J.S., Graham J.C., 2002. *Journal of Agriculture and Food Chemicals*, Vol.50 (22), pp. 6330.
11. Ghosal S., 1985. *Phytochemistry*, Vol. 24 (8), pp. 1807.
12. Singh S., Sanwal G.G., 1975. *Phytochemistry*, Vol. 14 (1), pp. 113.
13. Yue-Z.S., 1998. Recent natural products based drug development: a pharmaceutical industry perspective. *Journal of Natural Products*, Vol. 61, pp.1053–1071.
14. Hamburger M., Hostettmann K., 1991. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*, Vol. 30 (12), pp. 3864–3874.
15. Williamson E., Okpako D.T., Evans F.J., 1996. Selection, Preparation and Pharmacological Evaluation of Plant Material, Wiley, Chichester.
16. Goldfrank L., 1982. The Pernicious Panacea: Herbal Medicine. *Hospital Physician*,Vol. 10, pp. 64–86.
17. Soldati F., 1997. The registration of medicinal plant products, what quality of documentation should be required? The industrial point of view In: World Congress on Medicinal and Aromatic Plants for Human Welfare, Vol. 2, pp. L-48.
18. Vulto A.G., Smet P.A., 1988. In: Dukes, M.M.G. (Ed.). Meyler's Side Effects of Drugs, Elsevier, Amsterdam, 11th Ed. pp. 999–1005.
19. Gruenwald J., 1997. The market situation and marketing of herbal medicinal products (HMP) in Europe. In: World Congress on Medicinal And Aromatic Plants For Human Welfare, Vol. 2, pp. L-33.
20. Brevoort P., 1997. The current status of the US botanical market in: World Congress on Medicinal and Aromatic Plants For Human Welfare, Vol. 2, p. L-42.
21. Israelsen L.D., 1997. United States regulatory status of botanical preparations in:World Congress on Medicinal And AromaticPlants for Human Welfare, 2, pp. L-44.
22. Brevoort P., 1997. The current status of the US botanical market in World Congress on Medicinal and Aromatic Plants for Human Welfare, Vol. 2, pp. L-42.
23. Elisabetsky E., 1987. Pesquisas em Plantas medicinais. Cieˆncia e Cultura, Vol. 39 (8), pp. 697–702.
24. Rouhi A.M., 1997. Seeking drugs in natural products. Vol. 7, pp. 14–29.
25. Reid W.V., Laird S.A., Meyer C.A., Ga´mez R., Sittenfeld A., Janzen D.H., Gollin M.A., Juma C., 1993. Biodiversity Prospecting: Using Genetic Resources for Sustainable Development. World Resources Institute (USA).
26. Payne G., Bringi V., Prince C., Shuller M., 1991. The quest for commercial production of chemicals from plant cell culture,Plant Cell and Tissue Culture in Liquid Systems, Oxford University Press, Oxford.
27. Hamburger M., Hostettmann K., 1991. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*,Vol.30 (12), pp. 3864–3874.
28. Borris R.P., 1996. Natural products research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacology*, Vol. *51* (1/3), pp. 29–38.
29. Williamson E., Okpako D.T., Evans F.J., 1996. Selection, Preparation and Pharmacological Evaluation of Plant Material, Wiley, Chichester.
30. Petrovick P.R., 1997. Development of new drugs from plant origin. In: World Congress On Medicinal And Aromatic Plants For Human Welfare, Vol. 2, pp. L-21.
31. Arias T.D., 1999. Glosario de Medicamentos: desarollo, evaluacion y uso. Washington: Organizacio˜n Panamricana de La Salud. Organizacio˜n Mundial de La Salud, pp. 171.
32. WHO (World Health Organization), 1992. Quality control methods for medicinal plant materials, Geneva.
33. Leonard, DB. 2006. Medicine at Your Feet: Healing plants of the Hawaiian kingdom. pp.1-15.
34. Alexandra P.D., Monica G.M, Ronald E.W., Beatriz M.M., 2001. Food chemistry.Vol. 73 (3), pp. 327.
35. Adeyemi O.S., Oladiji A. T., 2009. *African Journal of Biotech*,Vol. 8 (5), pp. 858-859.
36. http://www.ayurveda-recipes.com/banana.html.
37. Kailash P., Varalakshmi P., 1992. Effect of banana stem juice on biochemical change in liver of normal and hyperoxaluric rats. *Indian Journal of Experimaental Biology*, Vol. 30, pp. 440-442.
38. Mayfield J., 1998. Diagnosis and classification of diabetes mellitus- *New Criteria. J. American academy of family physician*, Vol. 58 **(**8), pp. 11-15.
39. Rother K. I., 2007. Diabetes treatment: bridging the divide. *Journal of Medicine*, Vol. 356(15), pp. 1499-1501.
40. Lindstrom J., Peltonen M.,Eriksson J. G, Louheranta A., Fogelholm M., Uusitupa M., Tuomilehto J., 2006. High – fibre diet predicts long term weight loss and decreased type 2 diabetes risk; the Finnish diabetes prevention study. *Diabetologia*, Vol. 49, pp. 912 – 920.
41. Lamb R. E, 2006. Review of therapeutic groups for type 1 diabetes mellitus patients. *Journal of Diabetes and endocrine*, Vol. 3**(**2), pp. 11–12.
42. Dubois H.F., Bankauskaite V., 2005. Type 2 diabetes programme in Europe. *Journal of Euro Observe*,Vol. 7(2), pp. 5-6.
43. Razavi R., Chan Y., Afifiyan F. N., Liu X.J., Wan X., Yantha J., Tsui H., Tang L., Tsai S., Santamaria P.,.Driver J.P, Serreze D., Salter N. W., Dosch H. M., 2006. *Journal of Diabetes and endocrine*, Vol. 127(6), pp. 1123-35.
44. Eberhart M. S., Ogden C., Engelgau M., Cadwell B., Hedley A. A, Saydah S. H., 2004. Prevalence of Overweight and Obesity Among Adults with Diagnosed Diabetes-United States,*Morbidity Mortality Weekly Rep.* Vol. 53(45), pp. 1066-1068.
45. Harris M. I., Flegal K. M., Cowie C. C., Eberhardt M. S., Goldstein D. E., Little R. R., 1998. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults: *The Third National Health and Nutrition Examination Survey*. Diabetes Care, Vol. 21, pp. 18-24.
46. Burke J.P., Williams K., Narayan K.M.V., Liebson C., Haffner S.M., Stern M.P., 2003. A Population perspective on diabetes prevention; whom should we target for preventing Weight Gain? Diabetes care. Vol. 26, pp. 1999-2004.
47. Swanston F., Day S.K., Flatt C.K., Gould P.R., and Bailey C.J., 1989. Glycemic effects of traditional European plant Treatmentts for diabetes and Studies in normal and Steptozotocin diabetic mice. Vol.10, pp. 69-73.
48. Krentz A.J., Bailey C.J., 2005. Oral antidiabetic agents: current role in type2 diabetes mellitus. Vol. 65(3), pp. 385-411.
49. Nathan D.M., Buse J.B., Davidson M.B., Heine R.J., Holman R.R.,Sherwin R., 2006. Management of hyperglycemia in type 2 diabetes:A consensus algorithm for the initiation and adjustment of therapy:a consensus statement from the American Diabetes Associationand the European Association for the Study of Diabetes. *Diabetes Care*, Vol. 29(8), pp. 1963-1972.
50. Vedavathy S., Mrudala V., Sudhakar A., 1997. *Tribal Medicine of Chittoor District*, pp 56-58.

51. Suneetha B., Sujatha D., Prasad K V., 2010. Antidiabetic and Antioxidant Activities of Stem Juice of Musa Paradisiaca on Alloxan Induced Diabetic Rats,Pharmanest.*An InternationalJournal of Advances in Pharmaceutical Sciences*, Vol. 1 (2), pp. 167-176.
52. Piyush D., Kirtikar S., Tyagi M.K., Piyush G., Gambir J.K., Rimi S., 2012. Antidiabetic and Antihyperlipidemic effect of the stem of Musa sapientum in Streptozotocin – induced diabetic rats. *Journal of Diabetes*, Vol. 4 (4), pp. 378-385.
53. Dhanabal S.P., Sureshkumar M., Ramanathan M., Suresh B., 2005. Hypoglycemic effect of ethanolic extract of Musa sapientum on alloxan induced diabetic mellitus in rats and its relation with antioxidant potential. *Journal of Herbal Pharmacotherapy*, Vol. 5(2), pp.7-19.
54. Malik C.P., Singh M.B., 1980. PlantEnzymology and Histoenzymology, Kalyani Publishers, New Delhi, pp. 278.