Evaluation of *in vitro* anti-diabetic activity on ethanolic extract of aerial parts of *Murraya koenigii*

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ABSTRACT

Objective: The objective of the study is to open new avenues for the improvement of medicinal uses of *Murraya Koenigii* aerial parts for the selected area of antidiabetic activity.

Methods: Ethanolic extract of aerial parts of *Murraya Koenigii* were subjected for invitro anti diabetic activity studies by Non –enzymatic glycosylation of hemoglobin assay, glucose uptake in yeast cells, Alpha-Amylase inhibition assay.

Results: Extracts at different concentration possessed significant anti diabetic activity. At a concentration of 10mg/ml, it was highly effective as a standard ant diabetic drug.

Conclusion: Based upon the results it is clear that Murraya Koenigii aerial parts have significant anti-diabetic activity.

Key words: Ethanolic, Aerial Parts of *Murraya koenigii*, Antidiabetic activity,

INTRODUCTION

Diabetics is characterized by elevation of chronic glucose levels in blood because the body is unable to produce insulin, either because of impaired insulin secretion, impaired insulin action, or both. Chronic exposure to glucose level in blood is leading cause of renal failure, visual loss and a range of other types of tissue damage. It is a chronic disorder of pritein, carbohydrate, fat metabolism. It is a fast-growing global problem with huge social, health, and economic consequences. *Murraya Koenigii* is a sub-tropical, tropical tree belonging to family Rutaceaenative to Srilanka and India. The aerial parts of *Murraya koenigii* are also used as an herb in Ayurvedic medicine. They possess anti-diabetic, Antibacterial activity, Antifungal activity, Hypoglycemic activity Antiprotozoal activity, Antioxidant activity, Hematological studies, Hypolipidemic activity Anti-lipid per oxidative activity, Anti-Hypertensive activity, Respiratory disorders, Hepatoprotective and anti-ulcer activity, Anti-inflammatory activity, Anti-pyretic activity, Immunomodulatory activity, Anthelmintic activity, Diabetic induced renal damage Wound healing activity, Anti-cancer activity and Diabetic wound healing activity.

MATERIAL AND METHODS

Collection and identification: *Murraya Koenigii* aerial parts were collected from local area they were dried for 5-7 days under shade , segregated, pulverized by a grinderto fine powder prior to analysis. About 50gms of powdered *Murraya Koenigii* aerial parts was extracted with ethanol (250ml) by maceration process for a period of 5 days. The extract was concentrated to ¼ of its volume by using simple.

Preparation of *Murraya koeningii* stock solution: 0.1gm of *Murraya koeningii* extract was taken and dissolved in 100 ml of ethanol which is used as stock solution with the concentration of 10,mg/ml , from this stock solution, different concentration *viz.*, 2, 4,6,8 10, mg/ml were prepared using ethanol solution.

In-vitro anti-diabetic activity:

Non-enzymatic glycosylation hemoglobin assay: Antidiabetic activity of *Murraya koenigii* aerial parts was investigated by estimating the degree of non-enzymatic hemoglobin glycosylation, measured colorimetrically at 520 nm Glucose (2%), hemoglobin (0.06%) and gentamycin (0.02%) solution were prepared in phosphate buffer 0.01M, pH 7.4. 1 ml each of above solution was mixed. 2mg/ml 4mg/ml, 8mg/ml, 10mg/ml extract was added to above mixture. Mixture was kept in dark at room temperature for incubation for 72 hrs. At 520nm hemoglobin glycosylation was measured colorimetrically .The standard drug used for assay was alpha tocopherol. %inhibition was calculated.

Glucose uptake in yeast cells: Yeast cells solution was prepared by centrifugation (3,000xg; 5min) by using distilled water until clear solution was obtained and with the help of distilled water prepare 10 % (v/v) suspension. Various concentrations of extracts were added to 1ml of glucose solution (2mg/ml, 4mg/ml, 5mg/ml, 8mg/ml, 10 mg/ml) and incubate for 10 min at 37°C. Add 100µl of yeast suspension, mix well followed by incubation at 37ºC for 60 min. Followed by centrifugation (2500rpm for 5min), finally glucose level was estimated. Standard drug used for assay is Metformin.

Alpha amylase inhibition assay: Glycogen and starch yield glucose and maltose with the help of alpha

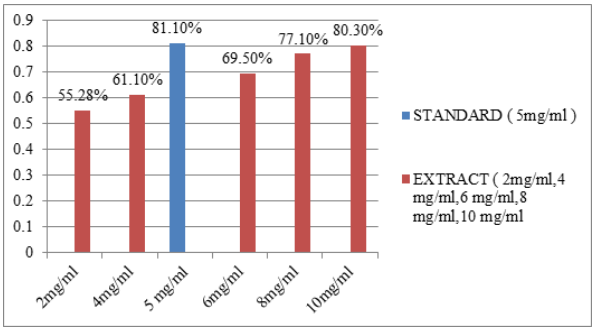
amylase. To 1ml substrate-potato starch (1%w/v), 1 ml of drug solution (metformin std drug, ethanol extract) of five different concentration such as 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml, 10 mg/ml. 1ml of alpha amylase (1%w/v) and 2ml of acetate buffer (0.1m, 7.2 pH) was added. NOTE: alpha amylase solution, drug solution, potato starch solution was prepared using acetate buffer. Followed by 1 hour incubation. Then add 0.1ml iodine iodide indicator (1 gm potassium iodide and 635 mg iodine in 250 ml distilled water). Note the absorbance at 565 nm in UV-visible spectroscopy %inhibition was calculated.

RESULT AND DISCUSSION

***In Vitro* Non enzymatic glycosylation of hemoglobin assay:** Human being minimizes the production of reactive oxygen species by enzymatic and non-enzymatic anti-oxidant mechanism, which plays a key role in many degenerative diseases including diabetes. High glucose levels in body leads to its binding to hemoglobin which may result in the production of reactive oxygen species. End products of glycosylation can be inhibited by plant extracts. Upon incubation of hemoglobin with different concentration of glucose over a period of 72 hour will increase glycosylation. However, upon increasing the concentration of hemoglobin the plant extracts inhibited hemoglobin glycosylation. *Murraya Koenigii* exhibited higher inhibition of glycosylation when compared with the standard drug. Over the period of 72 hour the plant extracts decreases hemoglobin glycosylation by decreasing the formation of the glucose-hemoglobin complex and amount of free hemoglobin increases. (Table 1, graph 1)

Table.1.Non-enzymatic glycosylation of hemoglobin assay

Blank	Standard			Ethanolic extract		
Abs	concentration	Abs	%inhibition	Concentration	Abs	%inhibition
0.1109	5mg/ml	0.5884	81.1%	2mg/ml	0.2480	55.28%
				4mg/ml	0.2856	61.1%
				6mg/ml	0.3647	69.5%
				8mg/ml	0.4854	77.1%
				10mg/ml	0.5647	80.3%



Graph.1.Non-enzymatic glycosylation of hemoglobin assay

***In vitro* glucose Uptake in yeast cells:** Control of blood sugar levels of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species. The present study indicates that the ethanolic extract possess good anti-diabetic activity. Glucose transport takes place through facilitated diffusion in yeast. Type 2 Diabetes is characterized by the deficiency of insulin causing increased amount of glucose in blood. After the treating yeast cells by these plant extract, the glucose uptake was found to increase as per dose. The results shows the % increase in glucose uptake by yeast cells at different glucose concentrations, the ethanol extract of 10mg/ml has showed significant activity when compared to the standard drug. (Table 2)

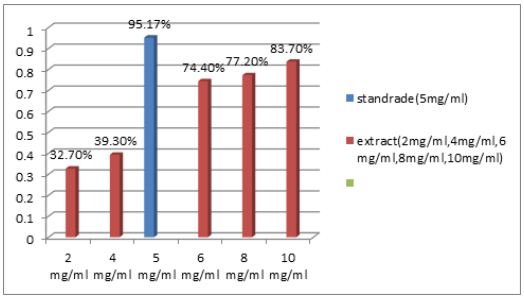
Table.2.Glucose uptake in yeast cells

Blank	Standard		Ethanolic extract	
Glucose concentration	Concentration	Glucose concentration	concentration	Glucose concentration
494mg/dl	5mg/ml	228mg/dl	2 mg/ml	484mg/dl
			4 mg/ml	382mg/dl
			6 mg/ml	320mg/dl
			8 mg/ml	285mg/dl
			10 mg/ml	235mg/dl

***In vitro* alpha amylase inhibition assay:** By the inhibition of alpha amylase enzyme post prandial glucose level in blood can be reduced. This may play a key role in management of blood glucose. The in-vitro α-amylase inhibitory studies demonstrated that *Murraya koenigii* aerial parts has good anti diabetic activity. The percentage inhibition at 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml, 10mg/ml. concentration of plant extracts shown concentration dependent reduction in percentage inhibition. At a concentration 2mg/ml,4mg/ml,6mg/ml,8mg/ml,10mg/ml. of *Murraya koenigii* aerial parts ethanol extract showed a percentage inhibition is 32.7%, 39.3%, 74.4%, 77.2%, 83.7%, respectively as per Table 3. As the result ethanolic extract of *Murraya koenigi* aerial parts shows significant activity when compared to metformin standard drug. (Table 3, graph 2)

Table.3.Alpha-Amylase inhibition activity

Blank	**Standard**			**Ethanolic extract**		
Abs	concentration	Abs	%inhibition	concentration	Abs	%inhibition
0.0088	5mg/ml	0.1824	95.17 %	2mg/ml	0.0130	32.7%
				4mg/ml	0.0145	39.3%
				6mg/ml	0.0345	74.4%
				8mg/ml	0.0386	77.2%
				10mg/ml	0.0540	83.7%



Graph.2.Alpha-Amylase inhibition method

CONCLUSION

In conclusion of our findings showed by *Murraya koenigii* showed to possess anti diabetic components. *Murraya koenigii* and its quantification of individual phytoconstituents as well as pharmacological profile based on in-vitro and in-vivo studies and on clinical trial should be further investigated.

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REFERENCES

1. Sen S, Chakraborty R, Biplab D, Challenges and opportunitiesin the advancement of herbal medicine. India's position and rolein a global context, J Herb Med, 1(1), 2011, 67-75.

2. Inesh Kumar, Mitra A and Manjunatha M, *In vitro* and in vivo studies of antidiabetic Indian plants. A review Journal of Herbal Medicine and Toxicology, 3(2), 2009, 0973-4643.

3. Arulselvan P, Senthilkumar GP, Sathish Kumar D, Subramanian S, Anti-diabetic effect of Murraya koenigii leaves on streptozotocin induced diabetic rats, Pharmazie, 1(10), 2006, 874–877.

4. Goutam MP, Purohit RM. Antimicrobial activity of the essential oil of the leaves of *Murraya koenigii*. Indian J. Pharm, 36, 1974, 11.

5. Narasimhan NS, Paradkar MV, Chitguppi VP, Kelkar SL, Alkaloids of *Murrayakoenigi*, Structures of mahanimbine, koenimbine, mahanine, koenine, koenigine and koenidine, Indian J. Chem, 13, 1975, 993.

6. Kishore N, Dubey NK, Tripathi RD, Singh SK. Fungitoxic activity of leaves of some higher plants, Natl. Acad. Sci. Lett, 5(1), 1982, 9-15.