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HYPOGYLCEMIC ACTIVITY OF AQUEOUS EXTRACT OF SOME INDIGENOUS PLANTS

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Pakistan is rich in medicinally important plants and has an ancient herbal treatment methods. Our work is based on the study of some indigenous plants which show inhibitory effect of glucose utilization, and are in use as hypoglycemic agent in traditional system of medicine. Gymnema sylvestre, Momordica charantia and Eugenia jumbolana have been shown to possess hypoglycemic activity of varying degree. The results in three different media revealed that, hypoglycemic activity is more prominent in neutral and basic media as compared to acidic medium.

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INTRODUCTION

A large number of plants used as food and medicine around the world are known for their ability to lower blood sugar levels through a variety of mechanism (Berger, 1985 and Maloff *et al.*, 1984).

Many compounds used in medicine are either derived directly from plants or as a synthesized form. Actually, many of the synthetic products are based on natural product (Rubatzky, 1997). These facts prompted us to initiate our work in search of such plants which could positively respond to common disorder of human i.e., Diabetes mellitus.

Gymnema sylvestre belongs to family Asclepiadaceae and is commonly known as Gurmar. Momordica charantia belongs to family cucurbitaceae and its common name is Karela and Bitter melon. Eugenia jumbolana is the member of family Myrtaceae and it is commonly known as Jaman.

EXPERIMENTAL

Materials

All the chemicals used in the experiments were AR grade. Spectronic 21 was used to record the results.

Methods

Method for the quantitative measurement of glucose in blood was introduced at the beginning of the century. Principal use of the test is for the diagnosis and management of diabetes (Alex *et al.*, 1983).

Two common clinical methods have been used for present work.

o-toluidine reagent

About 300ml glacial acetic acid was taken in 1liter volumetric flask. 1.5g thiourea and 9.5g sodium borate were added successively and 80ml o-toluidine was added to this solution. The volume was made up 1litre with glacial acetic acid (Jayashree, 1997).

Table 1Hypoglycemic effect of plants at pH 2 by o-toluidine method

S. No.	Name of plants	20mg/dl	40mg/dl	60mg/dl	80mg/dl	100mg/dl
1.	Gymnema sylvestre	20%	30%	35%	38%	40%
2.	Momordica C. (seed)	-	33%	38%	38%	38%
3.	Momordica C. (pulp)	-	33%	38%	38%	45%
4.	Eugenia J. (seed)	30%	48%	50%	51%	51%
5.	Eugenia J. (pulp)	25%	20%	20%	20%	20%

Table 2
Hypoglycemic effect of plants at pH 7 by o-toluidine method

S. No.	Name of plants	20mg/dl	40mg/dl	60mg/dl	80mg/dl	100mg/dl
1.	Gymnema sylvestre	50 %	50 %	50 %	55 %	60 %
2.	Momordica C. (seed)	45 %	60 %	61 %	64 %	65 %
3.	Momordica C. (pulp)	50 %	62 %	62 %	64 %	65 %
4.	Eugenia J. (seed)	17 %	35 %	36 %	40 %	40 %
5.	Eugenia J. (pulp)	-	16 %	28 %	28 %	30 %

Table 3 Hypoglycemic effect of plants at pH 9 by o-toluidine method

S. No.	Name of plants	20mg/dl	40mg/dl	60mg/dl	80mg/dl	100mg/dl
1.	Gymnema sylvestre	50 %	50 %	50 %	55 %	60 %
2.	Momordica C. (seed)	50%	50 %	55 %	55 %	57 %
3.	Momordica C. (pulp)	50%	55 %	55 %	60 %	61 %
4.	Eugenia J. (seed)	26 %	39 %	44 %	45 %	47 %
5.	Eugenia J. (pulp)	28 %	28 %	28 %	30 %	34 %

Preparation of glucose solution

0.25~g of glucose was dissolved in distilled water in 250 ml volumetric flask and filled the flask up to the mark. The concentration of this solution is 100~mg/100~ml.

To prepare 20, 40, 60, 80 mg/100 ml glucose solution took 20, 40, 60 & 80 ml from the above mentioned solution in 100 ml volumetric flask and distilled water added up to the mark. For acidic medium, all these solutions were made in 0.1M HCl and for basic medium pH9 has been maintained by using NaOH.

Aqueous extract of plants

5 g of specific parts of all mentioned plants were placed in separate conical flasks and 100 ml distilled water was added. The flask was heated on hot plate at 50°C for about 45 minutes and then filtered off in 100ml volumetric flask and the volume was made up to the mark.

Procedure for o-toluidine method

0.05 ml glucose solution of concentration 20, 40, 60, 80 & 100 mg/dl in different test tubes respectively were placed, then added 0.05 ml of plant extract in each test tube and kept for 4hrs. Then added 4 ml of o-toluidine reagent in all test tubes and were heated on boiling water bath for 15min. The test tubes were removed and cooled under running tap. The results were recorded at 630 nm after caliberation of instrument.

Procedure for glucose oxidase method

0.05 ml glucose solution of concentration 20, 40, 60, 80& 100 mg/dl in different test tubes respectively were placed, then added 0.05 ml of plant extract in each test tube and kept for 4hrs. Then added 5 ml of glucose oxidase enzyme in all test tubes and kept for 30 minutes in dark at room temperature. The caliberated spectrophotometer then adjusted at 546 nm wave length and results were recorded.

Formula for calculations

Conc. of glucose
$$= \frac{Au}{Ak} xC$$

where Au = Absorbance of unknown (extracts of plants)

Ak = Absorbance of known (standard glucose)

C= Concentration of standard glucose

RESULTS AND DISCUSSION

The results obtained from the present study have shown that at pH 7 and pH 9 a significant reduction in glucose concentration was recorded. All plants show varying degree of inhibitory effect on glucose utilization. The hypoglycemic effect of plants varies with the glucose concentration.

Gymnema sylvestre shows more hypoglycemic effect at pH=7 and pH=9 as compare to acidic medium at pH=2 i.e., 50-60% in neutral and basic media.

Momordica cahrantia seed and pulp show more or less same activity i.e. 50-65% at pH=7 and 9. It was also noted that hypoglycemic activity of unripe momordica seed and pulp is approximately same but when ripening of fruit increases the activity of seeds decreases and activity of pulp increases it may be because the pulp is quite bitter than seeds and have no sugar in it but seed also have its own sugar.

Eugenia jumbolana seeds have high hypoglycemic effect than its pulp.

We worked on two clinical methods i.e. o-toluidine method and glucose oxidase method. The results obtained from both methods are more or less same

Table 4
Hypoglycemic effect of plants at pH 7
by glucose oxidase method

S. No.	Name of plants	20mg/dl glucose
1.	Gymnema sylvestre	50%
2.	Momordica C. (seed)	40%
3.	Momordica C. (pulp)	50%
4.	Eugenia J. (seed)	40%
5.	Eugenia J. (pulp)	8%

REFERENCES

Alex Kaplan and Laverne L Szabo (1983). Clinical Chemistry Interpretation and Techniques. Lea and Febiger, Philadelphia, pp.311.

Berger W (1985). Incidence of severe side effects during therapy with sulfonylureas and biguanides. *Horm Metab Res Suppl.*, **15:** 111-115.

Jayashree Ghosh (1997). A Text Book of Pharmaceutical chemistry. 1st Edn., S. Chand & Company Ltd. pp.48.

Maloff BL, Drake L, Riedy DK and Lockwood DH (1984). Studies on hypoglycemic herbs. *Eur. J. Pharmacology*, **104**(3-4): 319-326.

Rubatzky VE (1997). "World Vegetables". 2nd Edn. International Thomson Publishing, pp.42.