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PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIDIABETIC ACTIVITY OF *MORINGA PTERIGOSPERMA* LEAVES IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The objective of this research was to isolate the flavonoids from the fresh leaves of *Moringa pterygosperma* Gaertn and evaluated for *in-vivo* antidiabetic activity of ethanolic extract and the ethyl acetate fraction on alloxan induced diabetic rat model.The fresh leaves of *Moringa pterygosperma Gaertn* were subjected to extraction with ethanol in a Soxhlet extractor for 10 hours. The ethanolic extract was fractionated using solvents of increasing polarity like Petroleum ether, benzene, chloroform, ethyl acetate and water. The preliminary phytochemical analysis of each extract revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins and glycosides as major components. The ethyl acetate fraction contains a high percentage of phenolics and flavonoids. The flavonoid isolated by preparative TLC was identified as rutin by HPTLC analysis. The ethanolic extract and ethyl acetate fraction were screened for hypoglycemic efficacy in alloxan induced diabetic rats. Both ethanolic extract and ethyl acetate fraction showed significant hypoglycemic activity as compared to standard drug, metformin. The result obtained in this research work indicated the promising effect of the hypoglycemic potential of ethanolic extract and ethyl acetate fraction as compared to standard metformin. These results clearly showed that flavonoids present in *Moringa pterygosperma* possesses potent hypoglycemic activity.

KEYWORDS: *Moringa pterygosperma* Gaertn, Soxhlet extractor, phenolics, flavonoid, rutin, TLC, HPTLC, hypoglycemic, Metformin.

INTRODUCTION

'Diabetes mellitus' describes a metabolic disorder of multiple etiologies and is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Because of the complications linked to diabetes like heart disease, retinopathy, kidney disease, and neuropathy, it also is a common cause of chronic morbidity and disability among the working population.[1] In traditional medicine diabetes mellitus is treated with diet, physical exercise and medicinal plants. Even though, more than 1200 plants are used around the world in the control of diabetes mellitus, only 30% of the traditionally used antidiabetic plants were pharmacologically and chemically investigated. On the other hand, potential hypoglycemic agents have also been detected from more than 100 plants used in antidiabetic therapy. Traditional treatments may provide the valuable clues for the development of new oral hypoglycemic agents and simple dietary adjuncts. More than 100 medicinal plants are mentioned in the Indian system of medicines, including folk medicines for the management of diabetes, which are effective either separately or in combination.[2]

Ayurveda and other traditional medical systems for the treatment of diabetes, describe a number of plants used as herbal drugs. Hence, they play an important role as an alternative medicine due to less side effects and low cost. The active principles present in medicinal plants have been reported to possess pancreatic β cells regenerating, insulin releasing and fighting the problem of insulin resistance.[3] *Moringa pterygosperma* is one of the widely used medicinal plants for the effective management of diabetes mellitus. All parts of *Moringa pterygosperma* viz. leaves, flowers, stem bark, roots have been proved to have medicinal value in which stem bark was proved to have antihyperglycemic activity.[4] But only limited studies have been conducted on leaves, the most edible part of *Moringa pterygosperma*. So the present study is focused on, screening the hypoglycemic activity of leaves of *Moringa pterygosperma*.

MATERIALS AND METHODS

Materials: The fresh leaves of *Moringa pterygosperma* belonging to the family Moringaceae were collected from Pala, Kerala, India. The plant was authenticated by Mr. Joby Paul, Botanist, Environmental Sciences, M. G University, Kottayam. India.

Extraction: The fresh leaves of *Moringa pterygosperma* (500 g) were exhaustively extracted with ethanol in a Soxhlet extractor for 10 hours.[5] The Marc (50 g) obtained after ethanolic extraction was subjected to aqueous extraction by reflux method. The extracts were concentrated to a solid residue and the yield was calculated.[6]

Fractionation of the ethanol extract: The ethanolic extract was fractionated using the solvents of increasing polarity like petroleum ether, benzene, chloroform and ethyl acetate. All the fractions were concentrated to a solid residue and calculated the yield.[7]

Animals: Male Wistar rats of 100-150 g and female Swiss Albino mice (20-25 g) were used for this study. The animals were obtained from the animal breeding station, Mannuthy, Trissur, Kerala. They were housed in ventilated cages and fed with pelleted diet and water ad libitum and maintained at 37°C and 12/12 hour light/dark cycle. The institutional animal ethical committee approved the research project.

TLC Analysis: Preliminary phytochemical analysis of total ethanolic extract and the ethyl acetate fraction showed the presence of flavonoids and tannins. Hence TLC analysis of ethanolic extract and the ethyl acetate fraction was done using different solvent systems were shown in table 1.

Table 1: TLC analysis of ethanolic extract and ethyl acetate fraction.

Solvent system	No. of spots	Detection
Toluene: Acetic acid	1 spot with tailing	UV light
Ethyl acetate: Formic acid: Glacial acetic acid: Water	**1 spot**	**Visible light**
Chloroform: Acetone: Formic acid	1 spot with tailing	UV light
Chloroform: Methanol	No spot was observed	Visible light
Ethyl acetate: Methanol: Water	3 spots	UV light
Toluene: Ethyl acetate	Tailing	Visible light
Toluene: Acetone: Formic acid	1 spot	Visible light

Isolation of Compound by Preparative TLC Method: Isolation of compound from an ethyl acetate fraction of *Moringa pterygosperma* leaves was carried out by preparative TLC method. Preparative TLC was done by using TLC plate of 20 x 20cm size. The solvent systems used are Ethyl acetate: Formic acid: Glacial acetic acid: Water in the ratio 100:11:11:26. After developing, the TLC plate was dried to obtain a yellowish brown spot. The separated compound in the plate was scraped out and transferred into a beaker. It was extracted with ethyl acetate and filtered. On concentration of the filtrate, a solid was

separated and this filtered and this was again subjected to TLC until a clear sharp spot of the compound was obtained.

High Performance Thin Layer Chromatography of Rutin: Based on the Rf value obtained by TLC analysis of the ethyl acetate fraction, HPTLC of the same extract was carried out to estimate the amount of rutin in the same extract.[8]

Reagents and standards

1. Rutin trihydrate (standard, Loba Cheme Pvt Ltd, Mumbai)
2. Methanol (We Associates, Kottayam)
3. Ethyl acetate (We Associates, Kottayam)
4. Formic acid (We Associates, Kottayam)
5. Glacial acetic acid (We Associates, Kottayam)
6. Purified water.

Preparation of standard: A stock solution of Rutin (100 µg/mL) was prepared in methanol. 10 mg of rutin was transferred into a standard flask and made up to 10 mL with methanol to get 1000 µg/mL solution. From this 1 mL was taken and made up to 10 mL to get working standard solution of rutin in the concentration of 100 µg/mL.

Preparation of sample: 20 mg of the Ethyl acetate fraction was dissolved in methanol in a standard flask and made up to 10 mL with methanol.

Stationary phase: HPTLC was carried out on pre-coated silica gel aluminium plate $60F_{254}$ (E Merck) of thickness 0.2 mm thickness and 10x10cm size.

Mobile phase: The mobile phase used was Ethyl acetate: Formic acid: Glacial acetic acid: Water in the ratio 100:11:11:26.[9]

Application of the sample solution: Various concentrations of the standard as 2, 4, 6, 8 and 10 µl were applied on HPTLC plates as bands using the spray-on-technique with a CAMAG Linomat V automatic sample applicator. Similarly, the ethyl acetate fraction of *Moringa pterygosperma* in two different concentrations as 20 and 30 µl were applied on the TLC plate. After application, the spots were dried using a dryer and the chromatogram was developed.

Development of the chromatogram:After sample application, the plates were developed in a CAMAG glass twin-trough chamber previously saturated with the solvent system Ethyl acetate: Formic acid: Glacial acetic acid: Water in the ratio 100: 11: 11: 26.

Detection: After development, the plates were dried and densitometric scanning was performed in CAMAG TLC scanner 3 at a wavelength of 254 nm without derivatization.

Antidiabetic Activity Studies

Acute toxicity study: Acute toxicity studies were carried out as per the guidelines 423 set by the Organization for Economic Cooperation and Development (OECD). Six female Swiss Albino mice weighing between 20-25 g were used for the study. They were fasted overnight and divided into 2 groups of 3 animals each.[10]

Group I - Ethanolic extract

Group II - Ethyl acetate fraction

The extracts were suspended in distilled water and administered orally (2000 mg/kg body weight). After the drug administration food was withheld for 2 hours. The animals were then observed continuously for mortality and behavior response during the first 30 minutes, periodically during the first 24 hours, and thereafter once daily for 14 days. Additionally, changes in the body weight and food consumption of the animals were observed. Changes in the body weight were recorded.

Antidiabetic activity in alloxan induced diabetic rats: Male Wistar rats weighing between 100-150 g were used. The diabetes was induced by the intra peritoneal (i.p) administration of Alloxan monohydrate (140 mg/kg body weight)[11] dissolved in normal saline. Forty eight hours after Alloxan administration, blood samples were drawn by retro orbital puncture and glucose level was determined by glucometer to confirm diabetes. The animal exhibits fasting blood glucose level greater than 150 mg/dl were selected for the studies.

Preparation of standard and sample: The standard used was Metformin. The standard (175 mg/kg body weight), ethanolic extract and ethyl acetate fraction (200 mg/kg body weight) were dissolved in distilled water and administered orally.

Antidiabetic activity: In this study total 30 animals were used in which 24 diabetic surviving rats and 6 normal rats. The animals were randomly divided into 5 groups of 6 animals each. Fasting blood glucose was checked in all groups of overnight fasted animals.[12]

Group I - Normal rats treated with vehicle alone (Negative control).

Group II - Diabetic rats (Diabetic control).

Group III - Diabetic rats treated with 175mg/kg body weight of standard (Metformin hydrochloride).

Group IV - Diabetic rats treated with 200mg/kg body weight of ethanolic extract.

Group V - Diabetic rats treated with 200mg/kg body weight of ethyl acetate fraction.

The standard and extracts were administered as single oral dose to group III, IV and V respectively.

After the administration of drugs, the blood was collected by retro orbital plexus puncture method[13] and blood glucose level was measured using a Glucocheck electronic glucometer. The blood glucose level was measured at 0^{th}, 1^{st}, 3^{rd}, 5^{th}, 7^{th} and 9^{th} h after drug administration. The blood glucose levels at various time intervals are recorded in table no: 5.

Statistical Analysis: The data are presented as mean ± SEM. Statistical comparisons between the groups were performed using Two Way ANOVA in Graphpad IV software. Data were considered significant when *p* values were lower than 0.05.

RESULT AND DISCUSSION

Based on the preliminary phytochemical analysis, further studies were carried out with the ethanolic extract and the ethyl acetate fraction of the *Moringa pterygosperma* revealed the presence of phenolics and flavonoids. The phenolics and flavonoids concentration are more in the above extracts when compared with other fractions. The flavonoid isolated by preparative TLC was identified as rutin by the same Rf value, and HPTLC analysis. The amount of rutin in the ethyl acetate fraction of *Moringa pterygosperma* was determined densitometrically by HPTLC. The linear range of rutin with regression coefficient 0.9955 was obtained. The amount of rutin in the ethyl acetate fraction was found to be 9.589 ng rutin/µg of extract as showed in figure 2.

Table 2: HPTLC analysis of Ethyl acetate fraction.

Amount (ng)	Rf	Area (ng)
Standard –Rutin		
200	0.37	1069.48
400	0.36	2205.29
600	0.36	3151.39
800	0.36	3820.69
1000	0.36	4666.16
Sample – Ethyl acetate fraction		
2000ng	0.33	383.56

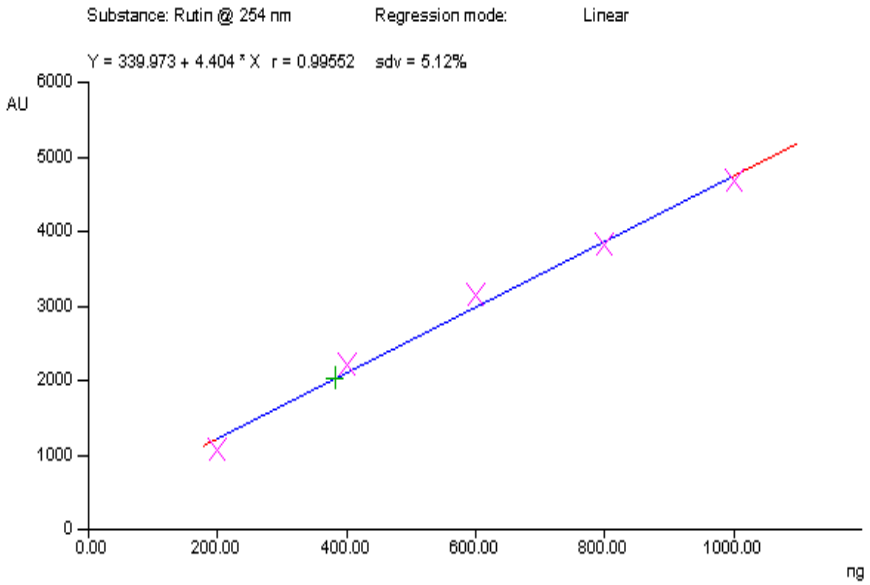

Fig. 1: Calibration curve of rutin.

Acute toxicity studies revealed that no mortality or any toxic symptoms and no change in food consumption were observed in sample treated animals. There was no significant change in the body weight as showed in table 3.

Table 3: Change in body weight of extract treated animals.

Treatment	Body weight in grams					
	0^{th} day	3^{rd} day	6^{th} day	9^{th} day	12^{th} day	14^{th} day
Ethanolic extract	20.66± 1.15	21.33± 1.52	22.0± 2.6	22.66± 2.8	23.0± 2.6	23.3± 1.5
Ethyl acetate fraction	21.33± 1.52	22.33± 2.51	23.33± 1.15	23.66± 1.52	24.0± 1.0	24.66± 0.57

Table no: 4 shows the hypoglycemic effect of ethanolic extract and ethyl acetate fraction on alloxan induced diabetic rat model. Group II shows that alloxan induced hyperglycemia in rats. There is a significant reduction in the blood glucose level in group IV and group V

treated with ethanolic extract and ethyl acetate fraction, respectively and is almost comparable to standard Metformin (group III).

Table 4: Blood glucose level at different time intervals.

Group of animals	Blood glucose level in mg/dl					
	0thhr	1sthr	3rdhr	5thhr	7thhr	9thhr
Group I (Negative control)	103.0±4.74	103.16±4.9	102.66±4.70	102.16±4.57	101.66±4.89	101.50±4.78
Group II (Diabetic control)	193.66±3.25	197.66±3.02	196.00±3.32	195.00±4.2	196.00±3.7	196.83±3.79
Group III (Standard)	190.16±4.57	176.30±5.54***	165.80±3.83***	157.50±3.83***	145.80±5.2***	138.50±5.03***
Group IV (Ethanolic extract)	189.50±4.44	181.50±3.97*	170.83±2.38***	162.16±2.86***	150.60±4.14***	140.50±3.44***
Group V (Ethyl acetate fraction)	188.16±3.51	180.60±4.12*	169.30±2.62***	158.30±2.59***	147.60±3.48***	138.83±2.39***

- *p< 0.05, *** p<0.001 when compared to 0 hr blood glucose level of the respective groups.

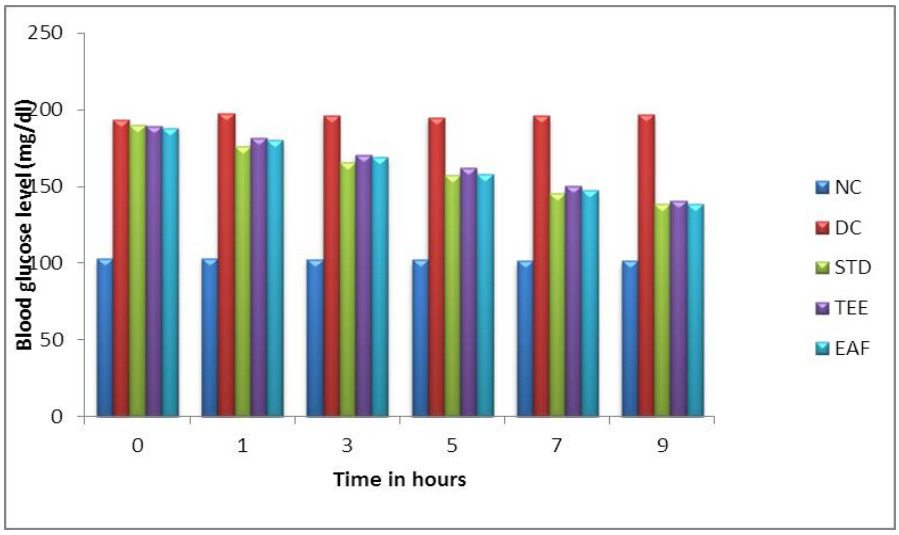

Fig. 2: Blood glucose level at different time intervals.

Karaca *et al.* has shown that dietary polyphenols play an important role in protecting the body against diabetes mellitus.[14] O Hnatyszn *et al.* has shown that the flavonoid "rutin" present in the *Phyllanthus sellowianus* Miller is responsible for the hypoglycemic of the plant.[15] So the antidiabetic activity of the *Moringa pterygosperma* leaves may be due to the flavonoid rutin present in it.

CONCLUSION

Both ethanolic extract and ethyl acetate fraction significantly reduced the blood glucose level in alloxan induced diabetic rats and it may be due to the antioxidant activity of the

polyphenolic compound present in the extracts. HPTLC analysis reveals that the compound present in the sample was found to be rutin. Since the rutin present in the *Phyllanthus sellowianus* has already shown antidiabetic activity,[16] the hypoglycemic activity in this case can attributed to the rutin isolated from the *Moringa pterygosperma* leaves. The above observation gives credence to the use of *Moringa pterygosperma* leaves as a hypoglycemic agent. Considering its safety, efficacy, easy availability, and popularity the leaves of *Moringa pterygosperma* offer a large scope for further development into antidiabetic herbal remedy.

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