Research Article

ANTIOXIDANT AND ANTIDIABETIC ACTIVITY ON ROOTS OF *SMILAX ZEYLANICA*

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ABSTRACT

Objective: To evaluate the antidiabetic and antioxidant activity on *Smilax zeylanica*. **Materials and methods:** The different extracts of *Smilax zeylanica* was studied for antidiabetic activity in streptozotocin induced diabetic rats by oral administration of extract 400mg/kg body weight for 21 days. The effect was compared with oral dose of 0.5mg/kg Glibenclamide and the extracts were also studied for antioxidant activity by DPPH radical scavenging activity. **Results:** The methanolic extract of *Smilax zeylanica* roots shows the better results as compared to other extracts and in phenol and Flavonoid content estimation methanolic extract showed the better results. The Phytochemical investigation showed the presence of phenol, flavonoid, glycosides, alkaloids Saponins. **Conclusion:** The existence of phenolic and flavonoid compounds in the extract may be accountable for the antidiabetic and antioxidant activity. Thus this activity can be contributed to the phytochemicals present in it. The methanolic extract of *Smilax zeylanica* can be concluded to possess highest amounts of phenolic, flavonoid and DPPH free radical scavenging activities from the present studies.

KEYWORDS: *Smilax zeylanica*, DPPH, Photochemical and Antidiabetic

INTRODUCTION

Diabetes is a growing epidemic around the world which is considered as a chronic incurable condition due to insulin deficiency that affects 10% of the population¹. The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025².

Drug treatment is not completely successful in the people with diabetes and there is a compelling need for better prevention and treatment strategies³. Studies revealed that about 75 % of patients use some of form of complementary and alternative medicine even though physician knowledge, regulatory strands and evidence of safety and benefit are often lacking.⁴

Diabetes mellitus isn't a solitary issue, it speaks to a progression of metabolic conditions related with hyperglycemia and caused by absconds in insulin discharge as well as insulin activity. Introduction to unending hyperglycemia may bring about micro vascular confusions in the retina, kidney and peripheral. Diabetes mellitus is a metabolic issue portrayed by the nearness of constant hyperglycemia joined by more prominent or lesser weakness in the digestion of sugars. In 1936, the refinement between type 1 and type 2 DM was unmistakably made. Type 2 diabetes was first depicted as a segment of metabolic disorder in 1988.

Smilax zeylanica belongs to the family *Smilacaceae*. It is a perennial climbing shrub which is locally known as Kumarilata and India smilax⁵. It has long history of use in the traditional medicine for the treatment of syphilis, gonorrhea, swelling, abscesses and boils. The roots of *Smilax zeylanica* contain Diosgenin, a steroidal saponins glycosides with medicinal value. Roots are eaten for the treatment of venereal diseases and skin troubles and a decoction of the bulbous root is given for sores, swellings and abscesses. Root also used in anemia, urinary disorders, blood dysentery, rheumatism⁶.

MATERIALS AND METHODS

Collection And Authentication Of The Materials

The plant *Smilax zeylanica* was collected in June from Yamuna biodiversity park, New Delhi in 2017. The formal authentication was done by Dr. Bhawana. S. Bajpai department of Botany Ch. Charan Singh University, Meerut. A voucher specimen [RUBL 21787]. The roots were allowed to air dry away from sunlight. The dried material was grounded coarsely to a powder and transferred to labeled brown bottles until required.

Extraction Of Plant Material

The coarse powder (90g) was extracted with petroleum ether, ethyl acetate and methanol, each 350ml in Soxhlet apparatus for 24hrs at 60°C. The collected mass was subjected to drying to evaporate the excess of solvent. The collected dark brownish color material was termed as petroleum ether, ethyl acetate and methanol extract of *Smilax zeylanica*.

Phytochemical Investigation

Chemical tests were carried out on all extracts for the qualitative determination of phytochemical constituents.

PHENOLIC CONTENT ESTIMATION

The total phenolic content was estimated by using Folin-Ciocalteu as indicated by the strategy for Singleton and Rossi⁷ with Gallic acid as the standard. Prepare standard stock solution (10mg/ml) of Gallic acid as the standard. From this various concentration ranging from 200-1000µg/ml will be prepared. To this 1 ml of Folin- Ciocalteu reagent (1:2 with water) will be added and kept at room temperature for 5min and then 1ml of 7% sodium carbonate solution will be added to the reaction mixture

and incubated at room temperature for 90 minutes. The color developed will be read at 750nm. A 100µl of methanol extract of sample will be mixed with the same reagents. Gallic acid is used as the reference standard and the results are expressed as milligram Gallic acid equivalent (mg/g) dry weight of plant material. All samples will be analyzed in triplicate.

FLAVONOIDS CONTENT ESTIMATION

Flavonoids content of drug extract was spectrophotometrically determined by the aluminum chloride method using quercetin as a standard according to the known method of Zhinsen et al⁸. The plant extract (0.1ml) was added to 0.3ml distilled water followed by 0.03ml NaNO₂ (5%) and incubated for 5 min at 25°C. Later 0.03ml of AlCl₃ (10%) was added and further after 5 minute, the reaction mixture was diluted to 1ml with water and the absorbance was measured at 510nm. The total flavonoids content is expressed as milligrams of quercetin equivalent (QE) to per gram of *Smilax zeylanica* extracts.

Determination of DPPH Radical scavenging activity

Principle – The antioxidant activity of all extracts was evaluated by the 2, 2 –diphenyl-1-picrylhydrazyl (DPPH) method. The odd electron in the DPPH free radical gives a solid assimilation most extreme at 517nm and is purple in shading. The shading abandons purple to yellow as the molar absorptive of DPPH radical at 517nm decreases as the odd electron of DPPH radical experiences blending with hydrogen from a free radical scavenging antioxidants to frame the diminished DPPH-H. The subsequent decolorization absorbance is created at 517nm has been utilized as a measure of radical scavenging activity⁹.

Preparation Of Reagents

DPPH Solution (100mM)

DPPH solution was set up by dissolving 33mg of DPPH in 100ml of methanol. From this stock solution, 10ml was taken and diluted to 100ml utilizing methanol to acquire 100mM DPPH solution and kept in a test tube covered with aluminum foil to protect from sunlight.

Ascorbic Acid Standard Solution

100µg/ml stock solution was prepared by dissolving 10mg of ascorbic acid in 100ml of distilled water, from this 10,20,40,60,80,100µg/ml were prepared.

Preparation Of Solutions

A stock solution of concentration 1mg/ml was prepared by adding 10mg of petroleum ether, ethyl acetate and methanol extracts in 10ml methanol and solutions of various concentrations of different extracts such as 50,100,200,400,800,1000µg/ml were prepared.

PROCEDURE

100µl of various concentrations (50-1000µg/ml) of different extracts and 100µl solution of DPPH was incubated at 37°C for 30 minutes and change in absorbance of reaction mixture was read at 517nm. An equal amount of methanol and DPPH was serves as control. The experiment was performed in triplicate and percentage radical scavenging activity was calculated by formula given below:

$$\% \text{ of inhibition} = \{ (A \text{ of control} - A \text{ of test}) / A \text{ of control} \} \times 100$$

PHARMACOLOGICAL STUDIES

Animals

Wistar rats (150-300g) were obtained from All India Institute of Medical Sciences New Delhi (India). The animals were housed in animal house of ITS College of Pharmacy, Muradnagar, Ghaziabad, India in polycarbonate in cages, in a room maintained under controlled room temperature 22±2°C, relative humidity 60-70% and provided standard pellet diet and water ad libitum. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Registration Number: **1044/PO/Re/S/07/CPCSEA**) and the care of laboratory animals were taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India. The animals were deprived of food for 24h before experimentation but allowed free access to water throughout. All studies were carried out by using six animals in one group for Anti-diabetic activity. The animals were ethically treated throughout experiment.

Antidiabetic Activity By Streptozotocin Induced Diabetes Model

Induction Of Diabetes In Animals

Streptozotocin is freshly prepared in 100mmol/citrate buffers, pH 4.5 male wistar rats weighing 150-200g fed with standard diet was injected with 60mg/kg streptozotocin i.p. Initially, blood glucose level is increased, reaching values of 150-200mg after 3h. 6-8 hr after streptozotocin, the serum insulin values are increased up to 4 times, resulting in a hypoglycemic phase which is followed by persistent hyperglycemia. After 72 hrs of streptozotocin administration, the blood glucose levels are measured and the rats showing blood glucose level > 250mg/dl are considered to be diabetic and are used in the study¹⁰.

Experimental Design

GROUPS	TREATMENT	DOSAGE (mg/kg)
I	Normal saline	0.5ml/kg
II	Normal saline + streptozotocin	0.5ml/kg + 60mg/kg
III	Petroleum ether extract + streptozotocin	400mg/kg
IV	Ethyl acetate extract + streptozotocin	400mg/kg
V	Methanol extract + streptozotocin	400mg/kg
VI	Glibenclamide + streptozotocin	5mg/kg

All animals are treated for 21 days. Glucose levels were measured on day 0 i.e. just prior to the initiation of any treatment on 7th day, 14th day and 21st day using one touch Glucometer. After 72hrs of streptozotocin administration, the rats showing blood glucose level > 250mg/dl are considered to be diabetic and are used in the studies. The animal was sacrificed through euthanasia.

HISTOPATHOLOGICAL EXAMINATION

Under anesthesia pancreas was carefully removed from rats and immediately placed in formalin solution. Tissues were prepared histologically and haematoxylin and eosin recoloring procedure was utilized.

STATISTICAL ANALYSIS

The factual investigation of the considerable number of results was completed utilizing one-way ANOVA tool after by Dennett's numerous correlation utilizing SPSS programming and the level of significance was determined in comparison with the control group.

RESULTS

Extraction

% yield = (weight of the concentrated extract obtained/ weight of the powdered drug taken initially) $\times 100$

Percentage yield of the extracts of *Smilax zeylanica*

<i>Smilax zeylanica</i> extracts	Amount of extract (in gm)	Percentage yield
Petroleum ether extract of <i>Smilax zeylanica</i>	1.78	1.99%
Ethyl acetate extract of <i>Smilax zeylanica</i>	2.04	2.26%
Methanol extract of <i>Smilax zeylanica</i>	3.72	4.13%

Phytochemical Screening

Phytochemical Screening Of The Extracts Of *Smilax zeylanica*

Phytoconstituents present	Petroleum ether extract of <i>Smilax zeylanica</i>	Ethyl acetate extract of <i>Smilax zeylanica</i>	Methanol extract of <i>Smilax zeylanica</i>
Alkaloids	(+)	(+)	(+)
Tannins	(+)	(+)	(+)
Saponins	(+)	(++)	(++)
Proteins	(-)	(+)	(+)
Flavonoids	(+)	(++)	(++)
Carbohydrates	(-)	(-)	(+)
Anthraquinones	(+)	(+)	(-)
Phenols	(+)	(+)	(+)
Steroids	(-)	(+)	(+)

Flavonoids Content Estimation

Total flavonoids content of standard (Quercetin)

Concentration ($\mu\text{g/ml}$)	Absorbance of standard (Quercetin)
100	0.286 $\pm$ 0.006
200	0.385 $\pm$ 0.091
400	0.534 $\pm$ 0.028
600	0.71 $\pm$ 0.05
800	0.871 $\pm$ 0.03
1000	0.998 $\pm$ 0.005

Standard curve of Quercetin

Estimation Of Flavonoid Content Of Different Extracts Of *Smilax zeylanica*

Concentration ($\mu\text{g/ml}$)	Absorbance of extracts		
	Petroleum ether	Ethyl acetate	Methanol
100	0.295 $\pm$ 0.003	0.241 $\pm$ 0.02	0.287 $\pm$ 0.017
200	0.306 $\pm$ 0.005	0.283 $\pm$ 0.05	0.385 $\pm$ 0.01
300	0.406 $\pm$ 0.005	0.385 $\pm$ 0.01	0.488 $\pm$ 0.008
400	0.694 $\pm$ 0.004	0.452 $\pm$ 0.03	0.698 $\pm$ 0.02
500	0.894 $\pm$ 0.005	0.652 $\pm$ 0.03	0.885 $\pm$ 0.02
600	0.904 $\pm$ 0.004	0.832 $\pm$ 0.03	0.913 $\pm$ 0.04

Total flavonoid content in petroleum ether extract of *Smilax zeylanica* (mg/g quercetin equivalent)

Total flavonoid content in ethyl acetate extract of *Smilax zeylanica* (mg/g quercetin equivalent)

Total flavonoid content in methanol extract of *Smilax zeylanica* (mg/g quercetin equivalent)

Total flavonoid content in different extracts of *Smilax zeylanica*

<i>Smilax zeylanica</i> extracts	Total flavonoid content (mg/g Quercetin equivalent)
Petroleum ether extract of <i>Smilax zeylanica</i>	21.06
Ethyl acetate extract of <i>Smilax zeylanica</i>	27.08
Methanol extract of <i>Smilax zeylanica</i>	32

Total Phenolic Content Estimation

Total phenolic content of Gallic acid (Standard)

Concentration (µg/ml)	Absorbance of standard (Gallic acid)
20	0.388±0.01
40	0.540±0.05
60	0.706±0.02
80	0.863±0.03
100	0.986±0.01

Data presented in mean ±SD, where n=3

Standard curve of Gallic acid

Estimation of phenolic content of different extracts of *Smilax zeylanica*

Concentration (µg/ml)	Absorbance of extracts		
	Petroleum ether	Ethyl acetate	Methanol
100	0.185±0.03	0.120±0.008	0.262±0.01
200	0.394±0.003	0.288±0.01	0.311±0.009
300	0.492±0.005	0.368±0.01	0.483±0.01
400	0.589±0.008	0.413±0.008	0.583±0.007
500	0.601±0.003	0.507±0.005	0.618±0.01
600	0.700±0.001	0.689±0.009	0.791±0.009

Total phenolic content in petroleum ether extract of *Smilax zeylanica* (mg/g Gallic acid equivalent)

Total phenolic content in ethyl acetate extract of *Smilax zeylanica* (mg/g Gallic acid equivalent)

Total phenolic content in methanol extract of *Smilax zeylanica* (mg/g Gallic acid equivalent)

Total phenolic content in different extracts of *Smilax zeylanica*

<i>Smilax zeylanica</i> extracts	Total phenolic content (mg/g Gallic acid)
Petroleum ether extract of <i>Smilax zeylanica</i>	16.93
Ethyl acetate extract of <i>Smilax zeylanica</i>	27.33
Methanol extract of <i>Smilax zeylanica</i>	34.44

DPPH Free Radical Scavenging Activity

DPPH percentage free radical- scavenging capacity for Ascorbic acid

Concentration ($\mu\text{g/ml}$)	Absorbance of standard (As)	Absorbance of control (Ac)	(Ac-As)/ Ac	[(Ac-As)/Ac] $\times 100$
10	0.299	0.419	0.286396	28.6396
20	0.257	0.419	0.386635	38.6635
30	0.202	0.419	0.5179	51.79
40	0.143	0.419	0.658711	65.8711
50	0.097	0.419	0.768496	76.8496

% Free radical- scavenging capacity DPPH activity

DPPH % free radical- scavenging capacity for standard & extracts of *Smilax zeylanica*

Concentration ($\mu\text{g/ml}$)	Ascorbic acid	Petroleum ether extract of <i>Smilax zeylanica</i>	Ethyl acetate extract of <i>Smilax zeylanica</i>	Methanol extract of <i>Smilax zeylanica</i>
10	28.6396	4.81	25.17	20.34
20	38.6635	12.56	30.46	31.89
30	51.79	23.53	36.87	44.09
40	65.8711	29.54	46.23	57.71
50	76.8496	34.76	51.74	69.43

In vitro Antioxidant activity of *Smilax zeylanica* by DPPH method

Antidiabetic Activity

Effect of different extracts of *Smilax zeylanica* blood glucose level against streptozotocin induced diabetic rats

Group	Treatment (mg/kg b.w.)	Blood glucose (mg/dl)			
		0 day	7day	14day	21day
I	Normal saline (0.5ml/kg)	89.16±5.7	86.96±3.9	85.88±4.5	83.78±4.8
II	Normal saline + streptozotocin (0.5ml/kg+60mg/kg)	276.7±9.5	267.8±8.3**	270.7±9.6**	259.0±14.6*
III	Petroleum ether extract + streptozotocin (400mg/kg)	217.8±10.1	209.2±9.3***	204.6±12.4***	194.7±9.8***
IV	Ethyl acetate extract + streptozotocin (400mg/kg)	214.5±8.1	150.7±5.4**	149.2±8.2**	145.7±7.5**
V	Methanol extract + streptozotocin (400mg/kg)	204.1±9.7	108.7±6.8**	122.6±8.4**	117.9±4.7**
VI	Glibenclamide + streptozotocin (5mg/kg+ 60mg/kg)	201.8±8.4	112.0±9.8***	111.7±10.8***	109.4±8.9***

All values are expressed as mean ± SD (n=5),

*p<0.001 when compared to control group

**p<0.002 when compared to standard (one way ANOVA followed by Dennett's test)

Histogram represents the effect of different extracts of *Smilax zeylanica* on blood glucose level against streptozotocin induced diabetes. Normal saline (group 1), Normal saline + streptozotocin (group 2), Petroleum ether extract + streptozotocin (group 3), Ethyl acetate extract + streptozotocin (group 4), Methanol extract + streptozotocin (group 5), Glibenclamide + streptozotocin (group 6)

Microscopic Evaluation By Histopathology
Antidiabetic Activity
Histopathological Examination Of Rat Pancreas

(Control group of *Smilax zeylanica*) arrow indicated the β -cells of islets of pancreas present in normal proportion

(Standard group of *Smilax zeylanica*) arrow indicated the destruction of β -cells of islet of pancreas

(Petroleum ether extract of *Smilax zeylanica*) arrow indicated the less destruction of β -cells of islet of pancreas. Cells are normal in shapes

(Ethyl acetate extract of *Smilax zeylanica*) arrow indicated the less destruction of β -cells of islet of pancreas

(Methanol extract of *Smilax zeylanica*) arrow indicated the normal shapes of β -cells of islet of pancreas. No destruction of cells.

(Glibenclamide group) arrow indicates the normal shapes of β -cells of islet of pancreas

CONCLUSION

The existence of phenolic and flavonoid compounds in the extract may be accountable for the antidiabetic activity and antioxidant activity. Thus this activity can be contributed to the phytochemical present in the extracts like alkaloids, glycosides, phenol, steroid, flavonoids and tannins. The methanolic extract of *Smilax zeylanica* can be concluded to possess highest amounts of phenolic, flavonoid and DPPH free radical scavenging activities from the present studies.

Therefore the antidiabetic and antioxidant activity of the extracts of *Smilax zeylanica* can be attributed to its phytochemical compound present in the extracts.

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