INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
2(1): January –March: (2012), 323-328
ISSN: 2231-6078

Research Article

Studies on the Physico-Phytochemical and Anti-diabetic Properties of *Cissus auadranaularis l.* and *Solanum torvum swartz.*

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Abstract

Cissus quadrangularis L. (Family: Vitaceae) and *Solanum torvum Swartz.* (Family: Solanaceae) are widely used in the native system of medicine for various ailments in Tamil Nadu. The hydroalcoholic extracts of these plants at dose levels of 200mg/kg body weight showed promising anti-diabetic activity in the Alloxan- induced model in rats. The overall anti-diabetic activity exhibited by the extracts is found to be low as compared to standard drug Glibenclamide. The preliminary Physico-phytochemical analysis carried out on the plants which revealed interesting results are highlighted and discussed.

Key Words: palmetto, quality control, disintegration time.

Introduction

Cissus quadrangularis L. (Family: Vitaceae) is known as Adamant creeper in English, Pirandai in Tamil. It is a perennial plant that belongs to the grape family and is popularly known as Veldt Grape Devil's Backbone, or Asthisamharaka.

It is distributed tropical in Africa, Namibia, South Africa (Transval, Natal, and Swaziland), Arabia, India, Ceylon, Thailand, Philippines. The rhizomes are useful for antioxidant, antimicrobial and as a lipid lowering agents in health and disease and also for the treatment of fracture bones, purgative and antidiabetic.

Solanum torvum Swartz (Family: Solanaceae) It is commonly known as Turkey berry, Eggplant or devil's fig[1]. It is also known as Sundaikkai in Tamil. *Solanum torvum swartz* is a small shrub, stout prickles, clearly petioled leaves with dense stellate hairs and native of Mexico, Peru and Venezuela. It is widely distributed in India, West Indies, Bermuda, Indonesia, Malaya, China, Philippines and tropical America[2-3]. The fruits are useful for treating liver and spleen enlargement, cough and haematopoietic, anti-microbial and analgesic [4-5]. Many valuable phytoconstituents of therapeutic importance such as steroidal alkaloids, chlorogenone, neochlorogenone, isoflavanoid sulfate and steroidal glycosides, 2,2 O-Spirostannol (Torvonin-A) have been earlier isolated classes of constituents reported [6-9]. Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins form increased risk factors leading to vascular diseases[10].

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The diverse medicinal properties of *cissus quandragularis L* and *Solanum torvum swartz* inspired us to investigate their anti-diabetic potentials. Administration of hydroalcoholic extract of *cissus quadrangularis* and *solanum torvum* produce significant reduction in the blood glucose levels. The possibility for this anti-diabetic action may be due to the presence of phytoconstituents similar to alkaloids, flavanoid types in the extract.

Available data on the anti-diabetic effect of these plants remain meagre**.** Against this backdrop, the present work was undertaken to throw move light on their pharmacological activities of the study plants.

Material and Methods

Collection of plant material

The rhizome of *cissus quadrangularis* and *solanum torvum* fruits were collected during the month of January and February from the delta areas located in and around Thanjavur district (TN). The collected specimens were authenticated by Botanist Dr. S. Rajan by comparing them with the herbarium specimen of Survey of Medicinal plants and collection unit (CCRH), Ooty. The voucher specimens were deposited in the herbarium of Department of Botany, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Tamil Nadu, India for future reference. The work was carried out in the Department of Pharmacology, Periyar college of Pharmaceutical sciences, Tiruchirappalli, Tamil Nadu, India. The clearance of Animal Ethical committee has been obtained from the college. The samples were washed with distilled water and dried under shade, mechanically pounded to get coarse powder and passed through number 40 sieve mesh. The sample powders were processed in such a way that they are useful for carrying out powder studies and phytochemical analysis.

Preparation of extracts

The coarse powder 150 grams of the given samples (*Cissus quadrangularis L* and *solanum torvum)* were extracted using 600 ml of hydroalcohol (20:80) by continous hot percolation with the help of soxhlet apparatus until the extraction procedure is complete. The successive extractions were done separately for each solvent namely, ethanol and water. The powder solvent ratio employed for the present study was 1:4. On completion, the extracts were filtered and the solvents were removed by distillation and dried under reduced pressure and controlled temperature 50º – 60ºC and refrigerated until use. The samples of the two extracts

were subjected to various analyses such as organoleptic characters[11], fluorescence studies[12], physico-chemical properties[13] and preliminary phytochemical screening[14-15]. The quantification of various metals present in the study samples were analysed using Atomic Absorption Spectrophotometer. The anti-diabetic activity of the hydroalcoholic extracts was evaluated as detailed below.

Evalution of Toxicity

The LD_{50} studies were carried out by Miller and Trainter method[16] and the results were reported.

Animal studies

Albino rats of either sex weighing 150 to 200 g belonging to Wistar strain were used in this work. The animals were acclimatized to the laboratory condition by subjecting them to dark and light cycles for 12 hours period before commencement of experiment. All the animals were given food and water ad libitum.

Evaluation of Anti-diabetic activity

- Group I served as control 2 ml/kg b.w (Normal saline)
- Group II served as a diabetic control (Alloxan induced)
- Group III received hydroalcoholic extract of *Cissus quangularis L* rizhome powder 200 mg/kg body weight.
- Group IV received hydroalcoholic extract of *Solanum torvum swartz* fruit (200 mg/kg body weight)
- Group V served as Reference standard drug Glibenclamide (5 mg/kg body weight)

Hyperglycemia was induced by a single intrapretonial injection of freshly prepared solution of Alloxan monohydrate (SD Fine Chemicals Pvt Ltd.) at a dose of 150mg/kg body weight were given for Group II, Group III, Group IV and Group V, whereas Group I received, similar volume of vehicle (Normal saline) 2 mg/kg bw. The hydroalcoholic extract of *Cissus quadrangularis L.* was administed for Group III, and *Solanum torvum swartz* hydroalcoholic extract for Group IV was administed orally by using catheter after Alloxan induction. For Group V, reference standard Glibenclamide drug was given orally. Treatment continued to fourteen consecutive days. After this period on fourteenth day Plasma glucose levels were estimated using Glucose oxidase method. The results were tabulated and analysed.

Histological studies: Diabetic animals were sacrificed at the fourteenth day after treatment and their pancreatic tissue were collected, washed with saline and placed in buffered formalin, followed by 70% alcohol solution for 24 hour. Tissue samples were fixed in Formalin and embedded in paraffin wax for light microscopic examination of HE sections[17] .They were shown in Figure1 and Figure 2.

Statistical analysis

Results were analysed as Mean ±S.E, n = 6. Data were tabulated and discussed using student't' test.

Results and discussion

Table 1-5 presents the results of physico-phytochemical study on the *Cissus quandrangularis L* (rhizome) and *solanum torvum swartz* (fruits). The rhizome and raw fruit (*Solanum torvum*) appear green in colour whereas dry fruits are orange red in colour respectively. *Cissus quandrangularis* rhizome has astringent taste with pungent odour and rough texture. *Solanum torvum* fruits are bitter in taste with no discernible odour and has coarse structure. These powders when viewed under UV light at 365 nm appear green *(Cissus quandrangularis*) and green (*Solanum torvum*). Under normal light, they are light green and green respectively. After treating with various biochemical reagents, they displayed narrow ranging colour variation. The hydroalcoholic extraction values are higher for *Solanum torvum* and water soluble extract values is higher for *Cissus quandrangularis* clearly indicate the presence of biological active constituents on the two extracts. The total ash value, acid insoluble value, sulphated ash value, moisture content values are higher for *Cissus quandrangularis* in comparision to *Solanum torvum*. Preliminary phyto-chemical screening of *Cissus quandrangularis* (rhizome) indicates the presence of alkaloids, flavanoids, tannins, proteins, carbohydrates, reducing sugars, gum and mucilage, whereas *Solanum torvum* (fruits) indicates the presence of alkaloids, flavanoids, saponins, proteins, carbohydrates, and reducing sugars. The alkaloid content is 2mg for *Cissus quandrangularis* and 1.25mg for *Solanum torvum* for 100 mg of sample. The flavanoid contents are present in equal quantity in the two extracts. Tannins show 0.6mg for *Cissus quandrangularis* and totally absent in the *Solanum torvum*.

Table 6 shows that elements like Fe, Cu, Zn, Mn, Cr and Mg value are higher for *Cissus quandrangularis* then *Solanum torvum*. Megnesium is predominant (306.60 ppm) for *Cissus quandrangularis* and (286.50 ppm) for *Solanum torvum.* Cr is present in lesser amount 9.84 ppm for *Cissus quandrangularis* and 9.31ppm for *Solanum torvum*.

Table 7 shows the LD_{50} values for both extracts which was found to be 2000 mg/kg body weight for *Cissus quandrangularis* and *Solanum torvum* respectively. One tenth of this value was chosen as initial dose in this work.

Table 8 shows the results of anti-diabetic activity of extracts which exhibited good anti-diabetic activity of the hydroalcoholic extracts of the study plants in the Alloxan-induced model used here. Diabetes induced by Alloxan was significantly reduced by the two extracts ($P < 0.001$) at the same dose levels of 200 mg/kg body weight of rhizome and fruits given. The rhizome of *Cissus quandrangularis* showed enhanced anti-diabetic acitivity than the fruit of *Solanum torvum*, which was evident from

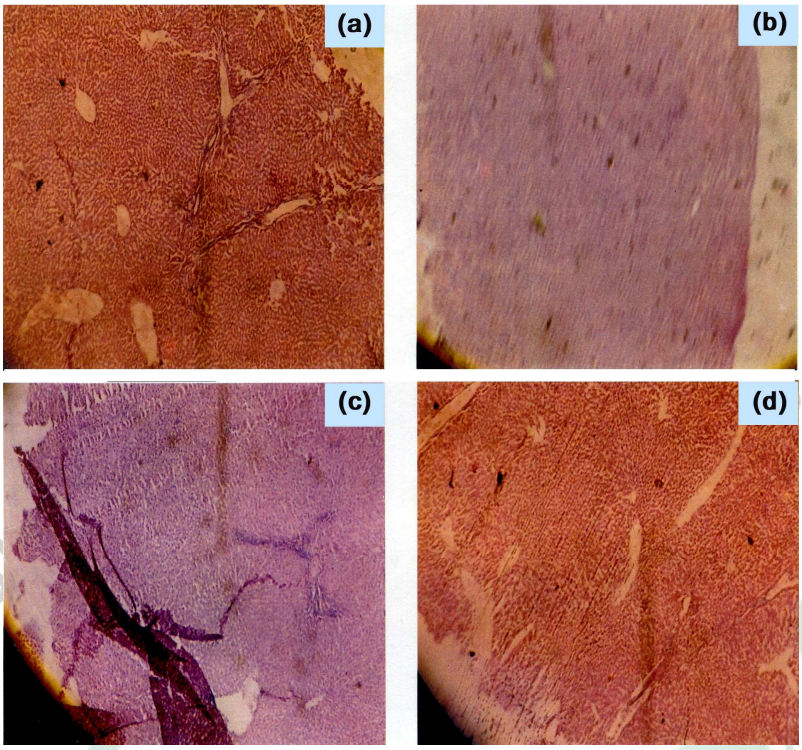

Fig.1. Histopathological studies on *Cissus quadrangularis L* (a) Normal saline at a dose of 2ml/kg, (b) Alloxan induced at a dose of 150 mg/kg, (c) Test sample induced at a dose of 200mg/kg, (d) Glibenclamide induced at a dose of 5 mg/kg.

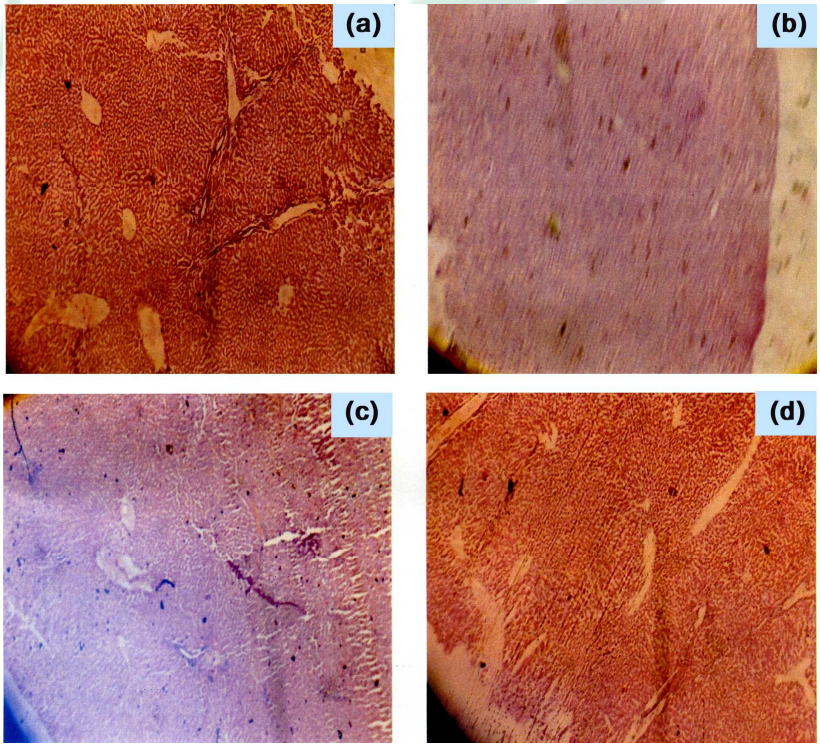

Fig.2. Histopathological studies on *Solanum torvum swartz* (a) Normal saline at a dose of 2ml/kg, (b) Alloxan induced at a dose of 150 mg/kg, (c) Test sample induced at a dose of 200mg/kg, (d) Glibenclamide induced at a dose of 5 mg/kg.

their respective plasma glucose level in mg% ($P < 0.001$) in comparision with diabetic control. However, the overall anti- adiabetic activity of the extracts was found to be much less than standard drug Glibenclamide. These preliminary finding await further studies on a larger set of data at different dose levels using more bio-chemical parameters[18]. The Histopathalogical studies on the pancreatic tissue before and after treatments have been done (Fig 1& 2). It records perceptible changes in the regeneration process of Pancreatic Tissue architecture.

Table 1: Organoleptic characters of *Cissus quadrangularis L* and *Solanum torvum swartz*.

Organoleptic Characters	*Cissus quadrangularis L*	*Solanum torvum swartz*
Colour	Green	Raw fruits appear green in colour. Dried fruits are orange red in colour
Taste	Astringent	Bitter
Odour	Pungent	Not Discernible
Texture	Rough	Coarse

Table 2: Fluorescence Studies of *Cissus quadrangularis L* and *Solanum torvum swartz*.

Characters	Daylight		UV light at 365nm	
	Cissus quadrangularis L	*Solanum torvum swartz*	*Cissus quadrangularis L*	*Solanum torvum swartz*
Sample	Light green	Green	Green	Green
Sample +1N Sodium hydroxide	Green	Greenish yellow	Greenish white	Greenish visible
Sample +1N Hydrochloric acid	Greenish	Green	Invisible	Invisible
Sample +50% sulphuric acid	Dark green	Green	Light green	Pale green

Table 3: Physico –chemical properties of Hydroalcoholic extracts of *Cissus quadrangularis L* and *Solanum torvum swartz*.

Characters	*Cissus quadrangularis L* (g) *	*Solanum torvum swartz* (g) *
Total ash	0.36	0.30
Water soluble ash	0.07	0.09
Acid insoluble ash	0.21	0.11
Sulphated ash	0.90	0.50
Moisture content	0.15	0.12
Alcohol soluble extractive	0.21	0.32
Water soluble extractive	0.34	0.26
Ether soluble extractive	0.15	0.11

*Estimation value for 1g of the sample.

Table 6: Quantification of various elements in the fruits of the *Cissus quadrangularis L* and *Solanum torvum swartz*.

Elements	*Cissus quadrangularis L* (ppm)	*Solanum torvum swartz* (ppm)
Fe	211.25	206.25
Cu	195.95	171.47
Zn	125.95	121.20
Mn	81.42	79.30
Cr	9.84	9.31
Mg	306.60	286.50

Summary and Conclusion

The anti-diabetic action may be due to suppression of transfer of glucose from the stomach to the small intestine and inhibition of glucose transport across the brush border of the small intestine[19-20]. From the above observations, it can be stated that the two extracts studied here displayed significant anti-diabetic activity at a dose of 200 mg/kg body weight in a model chosen for the present work. The *Cissus quandrangularis* rhizome extract exhibited more beneficial anti-diabetic modulating effect on plasma glucose level in Alloxan-induced diabetic rats than the fruits of *Solanum torvum swartz*. The present observations are not only in conformity with the traditional uses of these plants in the native system for treating diabetes but also provide a basis for examining the types of phytoconstituents responsible for anti-diabetic activity through future studies.

Acknowledgments

The authors are grateful to the Secretary and Correspondent, Principal, Dean of sciences and Head, Department of Chemistry, AVVM Sri Pushpam College (Autonomous), Poondi for their excellent encouragement and support.

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Table 4: Qualitative analysis of various phytoconstituents present in the hydroalcoholic extracts of *Cissus quadrangularis* and *Solanum torvum swartz*.

Phyto constitutents	*Cissus quadrangularis L*	*Solanum torvum swartz*
Alkaloids	(+)	(+)
Carbohydrates	(+)	(+)
Reducings sugars	(+)	(+)
Tannins	(+)	(-)
Flavanoids	(+)	(+)
Gums and mucilage	(+)	(-)
Saponins	(-)	(+)
Protein	(+)	(+)

(+) indicates Presence, (-) indicates Absence

Table 5: Quantitative analysis of various phytoconstituents present in the hydroalcoholic extracts of *Cissus quadrangularis* and *Solanum torvum swartz*.

Phyto constitutents	*Cissus quadrangularis L* (mg)	*Solanum torvum swartz* (mg)
Alkaloids	2.0	1.25
Carbohydrates	5.2	3.5
Reducings sugars	45.0	11.6
Tannins	0.6	0.0
Flavanoids	18.0	18.0
Gums and Mucilage	0.23	0.0
Saponins	0.0	0.31
Protein	9.8	8.0

***All values represent 100mg of the sample.**

Table 7: LD_{50} value of the hydroalcoholic extracts of two plants.

Test sample	LD_{50} value (mg / Kg. b.w)
Cissus quadrangularis L	2000
Solanum torvum swartz	2000

Table 8: Effect of *Cissus quadrangularis L* (rhizome) and *Solanum torvum swartz* (fruits) extracts on Plasma glucose levels.

Groups	Dose	Plasma glucose levels (mg %)	
		Cissus quadrangularis L	*Solanum torvum swartz*
Group –I control (Normal saline)	2 ml/kg	85.3 ± 3.1	85.3 ± 3.1
Group-II Diabetic control (Alloxan induced)	150 mg/kg	279 ± 9.4	279.1 ± 9.4
Group-III Test sample (*Cissus quadrangularis L)*	200 mg/kg	96.6 ± 3.4*	-
Group-IV Test sample (*Solanum torvum swartz)*	200 mg/kg	-	108.2 ± 2.8*
Group-V(Glibenclamide)	5 mg/kg	72.3 ± 8.2*	72.3 ± 8.2*

***Data are expressed as Mean ± S.E, n = 6**
*** P < 0.001 as compared to that of control by student't' test**