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NOTE

Phytochemical and Pharmacological Investigation of *Tinospora cordifolia* Miers

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Three extracts of *Tinospora cordifolia* have been prepared and evaluated for anti-diabetic activity. Ethyl acetate extract shows prominent activity when compared to the reference standard. An attempt is to isolate the active constituents responsible for hypoglycemic activity from ethyl acetate extract, five new phytoconstituents were isolated and their antidiabetic activity was studied. The active constituent's preliminary structure was elucidated by IR and mass spectra.

Key Words: Phytochemical, Pharmacological, *Tinospora cordifolia* Miers.

Tinospora cordifolia Miers (Menispermaceae) is widely distributed throughout India, also occurs in Burma and Ceylon. It is widely used in Ayurvedic medicine in India as tonic, vitalizer, as a remedy for diabetes mellitus and metabolic disorders[1-3]. Previous studies have shown that the stem exhibit antidiabetic[4], immunomodulatory[5], hepatoprotective[6] and antipyretic actions[7]. The report shows that leaves of *Tinospora cordifolia* having antidiabetic activity on Alloxan-induced diabetic rabbits[8]. The roots of *Tinospora cordifolia* possess antiulcer[9], antistress[10] and hypoglycaemic action[11]. It is reported that water and methanol extracts have antidiabetic activity. In the present study, various extracts of stems of *Tinospora cordifolia* have been evaluated for hypoglycaemic activity. In an effort to isolate active constituent(s) responsible for this activity, five new compounds were isolated which were not reported in literature[12-16] and the chemical structure of the potent one was characterized on the basis of IR and mass spectra.

Stems of *Tinospora cordifolia* were collected from indigeneous drug market of Nagpur. It was authenticated in Department of Botany, Nagpur University Campus, Nagpur. (The authentication number is Acc.No.7/1).

Preparation of the extract: Powdered stems were extracted with petroleum ether (60-80 ºC), the solvent was removed under vacuum and a crude solid mass was obtained. The marc was then re-extracted with benzene and the solvent was removed under vacuum and a crude mass was obtained. The marc after extraction with benzene was successively extracted with chloroform and ethyl acetate, respectively to get a solid crude mass. The dried crude extracts (petroleum ether, benzene, chloroform, ethyl acetate) were stored in a desiccator and used for further experiment after suspending in carboxy methyl cellulose (CMC) (0.5 % w/v) solution. The chemical constituents of the extract were identified by preliminary qualitative analysis and confirmed by thin layer chromatography (TLC) for the presence of terpenoids, sterols and saponins.

TLC of ethyl acetate extract: The ethyl acetate extract was subjected to TLC. Various solvent systems were tried, of which most suitable was $CHCl_3$:MeOH:CH_3COOH (9:1:0.1). The extract shows 5 spots which indicate number of constituents present in it.

Column chromatography of ethyl acetate extract: Ethyl acetate extract obtained from stems of *Tinospora cordifolia* was adsorbed on silica gel (60-120 mesh) from column chromatography. The slurry was air dried to remove any adsorbed moisture on surface and located on top of the column of silica gel packed with solvent system developed with the help of TLC technique. The constituent were separated using gradient elution technique. Separated constituents were subjected to Libermann-Burchard test for the presence of terpenoids.

Hypoglycemic activity: Various extracts, *viz.*, petroleum ether, benzene, chloroform and ethyl acetate extracts of *Tinospora cordifolia* were tested for hypoglycemic activity in Alloxan induced diabetic rats (Spraque Dawley rats).

The test compounds and the standard (metformin) drugs were administered in the form of a suspension (0.5 % CMC as a vehicle) in oral route at a dose levels 50, 100, 500 mg/kg animal body weight. Each group consists of 5 animals. The

TABLE-1
BLOOD SUGAR LEVELS AT VARIOUS TIME POINTS. SCREENING OF HYPOGLYCEMIC ACTIVITY OF DIFFERENT EXTRACTS

Group	Dose (mg/Kg)	Blood sugar level in mg/dl 1st day				Blood sugar level in mg/dl 3rd day	Blood sugar level in mg/dl 7th day
		0 h	1 h	3 h	5 h		
Control (saline)		275.92 ± 12.20	270.64 ± 3.20	268.96 ± 3.98	269.44 ± 6.32	202.66 ± 3.14	167.83 ± 2.36
Standard (metformin)	500	262.08 ± 8.18	218.43 ± 5.11	168.68 ± 4.01	135.98 ± 3.08*	119.92 ± 2.82*	98.34 ± 3.12*
Pet. ether extract	100	370.01 ± 4.21	368.09 ± 3.18	340.72 ± 2.98	364.52 ± 5.32	360.92 ± 3.09	369.47 ± 2.49
Chloroform extract	100	298.53 ± 3.28	287.63 ± 4.93	290.12 ± 3.06	293.02 ± 2.91	289.92 ± 2.58	282.09 ± 4.48
Benzene extract	100	315.60 ± 4.26	318.72 ± 5.86	298.05 ± 3.85	312.52 ± 4.62	305.42 ± 3.42	310.49 ± 3.09
Ethyl acetate extract	100	325.01 ± 5.91	298.05 ± 8.52	223.59 ± 3.82	192.56 ± 1.92	144.80 ± 2.91*	103.62 ± 3.49*

n = 6, values are mean ± standard deviation. *$p < 0.05$ with respect to corresponding control.

TABLE-2
RESULTS OF EVALUATION OF HYPOGLYCEMIC ACTIVITY OF SEPARATED CONSTITUENTS

Group	Dose (mg/kg)	Blood sugar level in mg/dl 1st day				Blood sugar level in mg/dl 3rd day	Blood sugar level in mg/dl 7th day
		0 h	1 h	3 h	5 h		
Control (saline)		275.92 ± 3.12	270.64 ± 1.08	268.96 ± 2.21	269.44 ± 3.02	252.68 ± 3.22	267.82 ± 9.28
Standard (metformin)	500	262.08 ± 1.16	218.43 ± 2.52	168.68 ± 9.16	135.98 ± 2.57*	119.92 ± 4.02*	98.34 ± 1.16*
Constituent-1	50	279.49 ± 3.10	250.82 ± 1.02	238.65 ± 3.21	245.86 ± 3.28	160.23 ± 3.82	150.72 ± 2.18
Constituent-2	50	289.87 ± 3.52	254.28 ± 2.15	202.46 ± 4.25	180.09 ± 2.01*	133.75 ± 4.29*	101.90 ± 2.81*
Constituent-3	50	270.52 ± 1.02	268.49 ± 4.68	255.79 ± 7.12	249.43 ± 5.48	225.49 ± 3.92	193.72 ± 2.42
Constituent-4	50	269.42 ± 4.02	265.29 ± 4.09	259.92 ± 3.92	248.49 ± 4.10	220.92 ± 5.09	149.42 ± 5.21
Constituent-5	50	284.52 ± 7.12	268.92 ± 5.12	249.42 ± 3.81	255.49 ± 2.92	236.42 ± 3.98	198.42 ± 4.08

n = 6, values are mean ± standard deviation, *$p < 0.05$ with respect to corresponding control.

animals were maintained in a colony cages at 25 ± 2 ºC, relative humidity of 45-55 %, maintained under 12 h light and dark cycle and were fed with standard animal feed. All the animals were acclimatized for a week before use. The Institutional Animal Ethics Committee has approved the experimental protocol.

Plasma glucose estimation by colorimetric methods: Each group of rats were subjected to fasting thereafter blood samples of each groups were collected from retroorbital plexus by means of sterilized glass capillary tubes under the ether anesthetic condition. The centrifuged plasma was separated from cold blood and used as sample. The glucose oxidase/ peroxides method was used for determination of plasma glucose levels in the rats. Per cent reduction in plasma/blood glucose level after administration of the extracts is shown in Table-1. The same procedure was adopted for determining reduction in plasma glucose level for isolated constituents and results are depicted in Table-2.

The stems of *Tinospora cardipholia* were collected, processed and extracted with various solvents and the crude extracts were subjected to the hypoglycemic activity by plasma glucose estimation by colorimetric method using Sprague Dawley rats. The results of hypoglycemic studies of crude extracts (Table-1) indicates that ethyl acetate extracts showed prominent activity when compared to standard metformin followed by other extracts. Hence, ethyl acetate extract was column chromatographed with the aim to isolate pure constituents responsible for hypoglycemic activity.

The isolated compounds were subjected to hypoglycemic activity (Table-2), the constituent 2 showed highest activity. In contrast, the hypoglycemic activity shown by ethyl acetate extract is more when compared to the isolated constituent 2. Due to its hypoglycemic activity, constituent 2 is further subjected to physiochemical studies which were shown below.

Constituent 2: Crystaline solid, m.p. 178 ºC, R_f value = 0.32, UV λ_{max} nm: 205, IR λ_{max} cm^{-1}: 3400, 2850-2975, 1750, 1050, 1025, 860, 650, 600. FAB-MS m/z: 601 (10), 428 (10), 398 (25), 289 (20), 241 (15), 154 (100), 150 (45), 138 (80), 120 (20), 109 (25), 108 (20). From the above chemical and spectroscopic data, it can be concluded that separated constituent 2 may be diterpenoid.

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