

Phytochemical Investigation and Hypoglycaemic Activity of *Tribulus terrestris*

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The flowers of *Tribulus terrestris* were analyzed phytochemically and the compound was isolated from extract. The compound was characterized by chemical and spectral methods and found to be quercetin 3-O-rhamnoside.

Key Words: Spectral studies, Hypoglycaemic activity.

INTRODUCTION

Tribulus terrestris Linn., belonging to Zygophyllaceae, is a prostrate herb with bright yellow flowers. The fruits constitute the commercial crude drug Gokshuru and are said to possess diuretic property. A paste prepared from the leaves is given for the treatment of stones in the bladder. An ethanolic extract of the fruits showed significant dose-dependent protection against uroliths induced by glass bead implantation in albino rats. It also protected leucocytosis and elevation in serum urea levels¹. Calcium, phosphorus and iron are reported to be present in the plants². Screening of the plant showed that it exhibited hepatoprotective activity³. The polyphenolic constituents of the petals have been investigated and its hypoglycaemic activity has also been examined.

Diabetes mellitus is one of the most widespread metabolic disorders in human beings and animals. It has been defined as a sustained state of hyperglycaemia and glycosuria⁵. Many plant species are known as folk-medicines of different cultures which have been used for their hypoglycaemic properties⁶. The efficacy of several indigenous plants of India alleged to exert hypoglycaemic effects has been reviewed^{7, 8}.

EXPERIMENTAL

The plant material was collected from Dharapuram of Erode district during September–October. The plant specimen was verified with the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

Extraction and fractionation⁴: The bright yellow coloured petals alone

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(1 kg) of *T. terrestris* were extracted with 80% methanol (5 × 500 mL) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was successively fractionated with light petrol (60–80°C) (3 × 250 mL), peroxide-free diethyl ether (3 × 250 mL) and ethyl acetate (5 × 250 mL). The ethyl acetate fraction was taken up for study.

EtOAc fraction (flavonol glycoside-quercetin 3-O-rhamnoside): The residue from EtOAc fraction was taken up in propanone and left in an ice-chest for two days when a pale yellow solid separated. It came out as pale yellow flakes on crystallization from methanol, m.p. 187–89°C, yield 0.1% and developed a greenish-brown colour with alc. FeCl₃ and reduced ammonical silver nitrate solution. It had λ^{MeOH} 254, 268, 358; +NaOMe 272, 327, 410; +AlCl₃ 275, 298, 433; +(AlCl₃-HCl) 268, 297, 362, 399; +NaOAc 263, 393; and +(NaOAc-H₃BO₃) 267, 300, 387 nm.

Acid hydrolysis of the glycoside: The glycoside (0.05 g) dissolved in hot aq. MeOH (2 mL, 50%) was hydrolyzed with 5% H₂SO₄ at 100°C for about 2 h. The excess alcohol was distilled off *in vacuo* and the resulting solution was extracted with diethyl ether.

Identification of aglycone (Quercetin): The residue from the diethyl ether fraction of the hydrolysate was taken up in propanone and left under chilled condition for a few days; a yellow solid was obtained. It was freely soluble in diethyl ether. It developed pink colour with Mg-HCl and an olive green colour with alc. Fe³⁺. It had λ^{MeOH} 255, 269, 370; +NaOMe 247, 306, 420; +AlCl₃ 272, 304, 333, 460; +(AlCl₃-HCl) 264, 303, 358, 426; +NaOAc 276, 329, 390; and +(NaOAc-H₃BO₃) 261, 303, 388 nm. Its R_f values matches with authentic sample of quercetin.

Identification of sugar (rhamnose): The filtrate after the removal of the aglycone was neutralized with barium carbonate. The concentrated filtrate when examined by paper chromatography showed R_f values corresponding to those of rhamnose. The identity of the sugar was confirmed by comparison with an authentic sample of rhamnose.

Hypoglycaemic activity

Swiss albino rats of either sex weighing between 140 and 180 g procured from King Institute of Preventive Medicine, Chennai were used for the present study. They were housed in microlon boxes and provided with water *ad libitum*. The animals were treated with 125 mg/kg of streptozotocin for induction of diabetes. Ten days later, the animals which shows blood sugar level above 250 mg were selected and divided into 4 groups each consisting of 6 animals. Six animals were (without streptozotocin treatment) kept for normal control. The group and the treatment were designated as Group-I streptozotocin control, Group-II *T. terrestris* 50 mg/Kg, Group-III *T. terrestris* 100 mg/kg and Group-IV *Tolbutamide* 50 mg/kg.

The drugs were given intraperitoneal route. Blood sample was collected from the animals' cardiac puncture at 1 h interval up to 3 h. The blood glucose was estimated by glucose-oxidase method (Glucometer-AMES).

RESULTS AND DISCUSSION

The fresh flower petals of *T. terrestris* have been found to contain quercetin (quercetin 3-O-rhamnoside).

The aglycone has λ^{MeOH} at 255, 269 and 370 nm and glycoside λ^{MeOH} 358 nm suggesting the presence of a flavonol skeleton in both⁹. A bathochromic shift of 50 and 52 nm in its NaOMe spectrum (band I) noticed in the aglycone and the glycoside indicated the presence of a free —OH at C-4'. The presence of a free —OH at C-5 in the aglycone and the glycoside is evident from its positive response to Wilson's boric acid test¹⁰. The same observation is also observed from the fact that a bathochromic shift of 56 and 41 nm was respectively in the aglycone and the glycoside in the $\text{AlCl}_3\text{-HCl}$ spectra¹¹. A shift of 7 and 9 nm noticed in the (band II) NaOAc spectra of the aglycone and glycoside indicated the presence of a free —OH at C-7. The presence of O-dihydroxyl group in the B-ring of the aglycone could be inferred from an additional bathochromic shift of 18 nm (band I) in the NaOAc- H_3BO_3 spectrum confirming the O-dihydroxyl group in the B-ring (C-3' and C-4'). A bathochromic shift of 29 nm (band I) of the glycoside in its NaOAc- H_3BO_3 spectrum showed the presence of catechol type of substitution in the B-ring. A bathochromic shift observed in methanol spectrum (band I) of the aglycone obtained on hydrolysis of the glycoside suggested the site of glycosylation to be at C-3. It was also supported by the fact that the glycoside did not respond to the Horhammer-Hansel test.

In the ^1H NMR spectrum (500 MHz, DMSO-d_6 , TMS) of the glycoside, the C-5' proton appears as a doublet at δ 7.10 ppm. The signals due to the protons at C-2' and C-6' are observable at δ 7.48 and 7.75 ppm, respectively. A ring proton at C-6 could be located at δ 6.2 ppm and a C-8 proton at δ 6.6 ppm. The signals at δ 12.6 and 10.8 ppm can be traced to the —OH at C-5 and C-7. The H-1'' of rhamnose resonates at δ 5.48 ppm. The rest of the sugar protons appear in the region between δ 3.00 and 4.00 ppm.

Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C NMR (500 MHz, DMSO-d_6 , TMS) and a complete assignment of the signals to various carbons is justified. Due to glycosylation, the signal of C-3 is shifted by 3.80 ppm¹². The downfield shift of the ortho related C-2 signal by 4.90 ppm also confirms this¹³.

The effect of flavonoids from *T. terrestris* on streptozotocin induced blood glucose method, the blood glucose level shows dose dependent hypoglycaemic activity at tested doses. The maximum activity (55.2%) was observed 3 h after the administration of the test substance which was comparable to that of oral hypoglycaemic activity of a standard drug Tolbutamide (50 mg/kg) (Table-1).

TABLE-I
EFFECT OF FLAVONOIDS ON HYPOGLYCAEMIC ACTIVITY

Group	Dose (mg/kg BW)	Decrease in blood glucose level				% Activity at 3 h
		0 h	1 h	2 h	3 h	
Normal	—	• 90.4	90.4	90.4	90.4	—
<i>T.terrestris</i>	50	258.6 ^a (±4.32)	182.1 ^a (±2.17)	136.4 ^b (±2.17)	120.8 ^a (±3.26)	50.5
	100	258.4 ^a (±4.32)	166.7 ^a (±2.17)	128.4 ^b (±2.17)	115.8 ^a (±2.36)	55.2
Tolbutamide	50	270.2 ^a (±1.42)	140.6 ^a (±1.53)	130.2 ^a (±2.03)	100.1 ^c (±2.32)	63.0

Values are mean ± SE of 6 animals.

Statistically significant from control group, where

^aP < 0.001, ^bP < 0.01, ^cP < 0.05

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