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Anti-Inflammatory Activity of Tricetin-7-O-Galactoside from Hypochoeris Glabra

KEYWORDS: *Hypochoeris glabra*, tricetin – 7 – O – galactoside, anti-inflammatory, SRBC membrane stabilization.

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ABSTRACT *The fresh flowers of Hypochoeris glabra is a shrub belonging to Asteraceae family have been found to contain tricetin –7–O– galactoside.The structure of the above compounds have been ascertained by paper chromatography,UV, 1H NMR and 13C NMR spectral values .The glycoside isolated from the flowers of Hypochoeris glabra has antioxidant property these components were used as inhibitors of the drug induced lipid peroxidation.*

Introduction

Hypochoeris glabra Linn. of composite is an annual plant of Travancore hills at altitudes of 4000 – 8000ft; it has also been recorded form Aka hills in Assam.The fresh herb possesses vulnerary properties. Leaves are astringent. The root is tonic, aperients and diuretic.In the absence of any flavonoidal work in the flowers of *Hypochoeris glabra* fresh flowers of the same has been chosen to investigate its phytochemical and pharmacological properties.

Experimental method

Extraction and Fractionation

Fresh flowers of ***Hypochoeris glabra*** (1Kg) collected during november from the kodaikanal were extracted with 80% MeOH under reflux. The alcoholic concentrate was successively fractionated with benzene(3x250), peroxide-free Et_2O (3x250ml) and EtOAc (4x250). No crystallisable solid could be obtained from benzene fraction.

Et_2O Fraction: (Flavone-Tricetin)

The yellow residue obtained from the fraction afforded yellow crystals which appeared deep purple under UV which turned yellow on exposure to ammonia. It answers Gibb's and wilson's boric acid tests but did not respond to Horhammer-Hansel and Molisch's tests. It is soluble in EtOH,Et_2O, but insoluble in water, benzene, free Et_2O. It had λ_{max}^{MeOH}nm 248, 265,300sh,348;+NaOMe 250, 266sh, 301sh,393;+$AlCl_3$ 248, 266, 299sh, 433; +$AlCl_3$ / HCl 250, 270, 300 sh, 403; + NaOAc 272, 302sh, 350;+NaOAc/ H_3BO_3 250, 271, 300sh 360; It had R_f values as depicted in Table I-1. It was identified as tricetin, in comparisons with authentic sample.

EtOAcFraction: (Tricetin– 7 – *O*-galactroside)

The ethylacetate fraction yielded yellow solid which on recrystallisation gave yellow flakes. It is soluble in water, ethyl methyl ketone and EtOAc. It is insoluble in benzene, Et_2O. It answered Gibb's, wilson's boric acid and Molish's tests. It never respond to Horhammer-Hansel test. It had λ_{max}^{MeOH}nm 271, 308sh, 340; + NaOMe 278, 300sh, 386; $AlCl_3$ 253sh, 289, 298, 498; +$AlCl_3$/HCl 280, 300sh, 340, 375; + NaOAc 278, 298sh, 365; + NaOAc/H_3BO_3 281, 300sh, 355;. It appeared deep purple under UV with or without NH_3.It had R_f values as depicted in Table I – 1. It was identified as tricetin -7- *O*- galactoside by observing its ^1H and ^{13}C-NMR values.

Hydrolysis of the glycoside

To a solution of the glycoside (0.05) in hot MeOH (2M) an equal volume of H_2SO_4 (7%) was added and the mixture gently refluxed at 100°C for 2h. the excess of alcohol was distilled off ***in-vacuuo*** and the resulting aqueous solution was extracted with Et_2O. The residue from Et_2O fraction was studied as described below.

Identification of the aglycone (Tricetin)

The residue from Et_2O fraction was taken up in Me_2CO and left under chilled condition for a few days when an yellow solid was obtained. It was subjected to colour reactions, PC and UV spectral studies as described under ether fraction.

Identification of the sugar (Galactose)

The aqueous filtrate and the washings of Et_2O layer were collected in a standard flask and the solution made up to the mark. The aqueous filtrate was neutralised with $BaCO_3$ and the concentrated filtrate was subjected to PC. The $R_f$values (Table I–2) corresponded galactose. The identify of the sugar was also confirmed by direct comparison with the authentic sample. The flavonoid glycoside has been characterized as tricetin -7-*O*- galactoside.

TABLE I-1
R_f(X100) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF HYPOCHOERIS GLABRA
(Whatmann No.1; Ascending 30+2°C)

	Developingsolvents*							
Compound	a	B	c	d	e	F	g	h
Aglycone from Et2O fraction	3	5	16	30	60	56	28	37
Tricetin (authentic)	3	5	16	30	60	56	28	37
Flavones glycoside from EtOAc fraction	37	8	17	35	70	58	65	80
Tricetin -7-*O*-galactoside (authentic)	37	8	17	35	70	58	65	80

*Solvent key

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a-H_2O
b-5 % aq.HOAc
c-15% aq.HOAc
d-30% aq.HOAc
e-60% aq.HOAc
f-t-BuOH :HOAc : H_2O = 4:1:5 (Upper phase)
g-phenol saturated with water
h-t-BuOH :HOAc : H_2O =3:1:1
i=HOAc :con.HCl: H_2O =30 :3:10

TABLE I-2
R_f(X100) VALUES OF SUGAR FROM THE GLYCOSIDE OF HYPOCHOERIS GLABRA
(Whatmann No.1; Ascending 30+2°C)

Compound	Developing solvents*			
	e	f	g	h
Sugar from the hydroysate of EtOAc fraction	73	09	44	89
Galactose (authentic)	73	09	44	89

TABLE I-3
^{13}C-NMR DATA AND THEIR ASSIGNMENT FOR THE GLYCOSIDE FROM THE FLOWER OF HYPOCHOERIS GLABRA

Compound	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Glycoside (δ ppm)	163.2	102.8	181.8	163.2	130.4	163.2	93.5	149.7	102.8
Compound	C_1'	C_2'	C_3'	C_4'	C_5'	C_6'			
Glycoside (δ ppm)	121.5	102.8	145.8	121.5	145.8	102.8			
Compound	C_1''	C_2''	C_3''	C_4''	C_5''	C_6''			
Glycoside (δ ppm)	73.1	70.6	78.9	70.3	81.5	61.5			

ANTI-INFLAMMATORY ACTIVITY
SRBC MEMBRANE STABILIZATION STUDIES
COLLECTION OF BLOOD:
Blood was collected from Sheep. The collected blood was mixed with equal volume of sterilized Alsever solution (containing 2% dextrose, 0.87 Sodium citrate, 0.05% Citric acid and 0.427% Sodium chloride) and stored at 4°c.

SALINE :
Saline at different concentration was prepared (Isosaline 0.85% and hyposaline 0.25%).

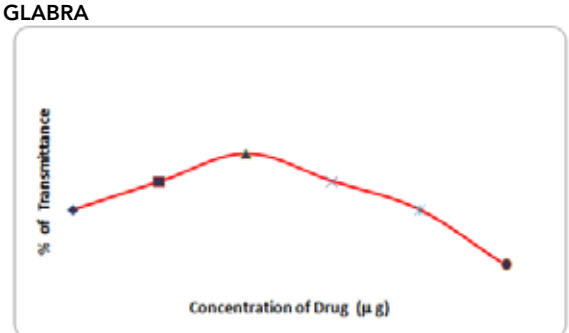
PREPARATION OF SRBC SUSPENSION :
The blood was centrifuged at 3000 rpm and the packed cells obtained were washed with isosaline (0.85%, pH-7.2) 3 times and a 10% (v/v) suspension was made with isosaline.

DETERMINATION OF SRBC MEMBRANE STABILIZATION :
Solutions of different concentrations of flavonoid are prepared. Assay mixture contained the drug (Flavonoid in concentrations are mentioned in Table I-4). 1 ml of Phosphate buffer (0.5% pH-7.4) 2ml of hyposaline (0.25%) and 0.5ml of 10% SRBC suspension. In another tube, instead of drug, 2ml distilled water was taken and this served as the control. All the tubes were incubated at 37°c for 30 minutes. Then they were centrifuged and the haemoglobin content in the supernatant was estimated using a photoelectric colorimeter at 560nm.

Table – 1-4
SRBC MEMBRANE STABILIZATION OF HYPOCHOERIS GLABRA

S.NO	CONCENTRATION OF DRUG(µg	TRANSMITTANCE (%)
1	10	18
2	25	19
3	50	20
4	75	19
5	100	18
6	200	16

SRBC MEMBRANE STABILIZATION OFHYPOCHOERIS GLABRA



RESULTS AND DISCUSSION
The **SRBC** membrane stabilization studies is used as a screening study for the anti-inflammatory activity of the isolated Tricetin – 7 – *O* – galactoside from Hypochoeris glabra.While the concentration is increased from 10 g the capability is also increases with in concentration. Thus initially it was observed that the capacity is a dose dependent one. But after reaching an optimum stabilization at 50 g the trend declines for further increase in concentration. That is beyond the concentration of 50 g only hypotonicity induced haemolysis is observed. Such kind of biphasic property is common in flavonoid glycosides.

Conclusion
From the trend observed in the foregoing experiment, it is observed that the drug tricetin only destabilize the membrane thus the invitro study was shown that the galactoside is observed to have hypotonicity induced haemolysis alone.

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