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Antioxidant activity of opuntia stricta

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ABSTRACT

The fresh flowers of *Opuntia stricta* Haw syn. *O.dillenii* Haw is a shrub belonging to Cactaceae family have been found to contain Rhamnetin and its glycoside Rhamnetin-3-O-rutinoside. The structure of the above compounds have been ascertained by paper chromatography, UV, C13 NMR and H1 NMR spectral values. The glycoside isolated from the flowers of *Opuntia stricta* has antioxidant property. These components were used as inhibitors of drug induced lipid peroxidation.

Keywords : *Opuntia stricta*, Rhamnetin-3-O-rutinoside-antioxidant- thiobarbituric

1. Introduction:

Opuntia stricta Haw syn. *O.dillenii* Haw is a shrub belonging to Cactaceae family. It is popularly known as Pathala mooli in Tamil. The flowers are usually yellow in color found throughout India. The flowers contain the glycoside of Isorhamnetin and quercetin in the proportion of 3:1 with small amounts of free flavonols. The mucilage contains galactose, glucose and arabinose. It may be used as a drier in white or color wash. The backed fruit is said to be given in whooping cough and syrup of the fruit is set to increase the secretion of bile and control spasmodic cough and expectoration.

2. Experimental:

2.1 Extraction and fractionation

The fresh flower petals (2 kg) of *opuntia stricta* collected at Kumbakonam of Thanjavur District during December. They were extracted with 85% methanol (5X500 ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with Benzene (3X250 ml) peroxide free ether (2X250 ml) and EtOAc (8X250 ml). Only EtOAc fraction was taken up for study. This Et2O fraction yielded Rhamnetin and EtOAc fraction yielded Rhamnetin-3-O-rutinoside.

2.2 Characterization;

Rhamnetin : Yellow solid, M.p 290-294°C . It had λ_{max} MeOH 256,271sh,300 sh,370; +NaOMe 245sh,321,410 ;+AlCl₃ 272,304 sh,332,457;+AlCl₃-HCl 265,301 sh, 328,417;NaOAc 272,330,389 and +NaOAc-H₃BO₃ 260,302 sh,392nm. The Rf values of the aglycone are tabulated in Table 1.3. It was identified as Rhamnetin and the identity was confirmed by mixed PC with an authentic sample of Rhamnetin1.

Rhamnetin-3-O-rutinoside: yellow colour solid, M.p 265-270°c. It had λ_{max} Me OH 256,265 sh,302 sh,355;+NaOMe 270,329,405;+AlCl₃ 273,303 sh,432;+AlCl₃-HCl 268,303 sh,364,401;+NaOAc 257 and + NaOAc-H³BO³ 260;298 sh. It had Rf values obtained for Rhamnetin-3-O-rutinoside are recorded in Table 1.1&1.2. C13NMR, H1 NMR values are illustrated in Table 1.3.This was confirmed by direct comparison with an authentic sample of *Suriana maritime* 2.

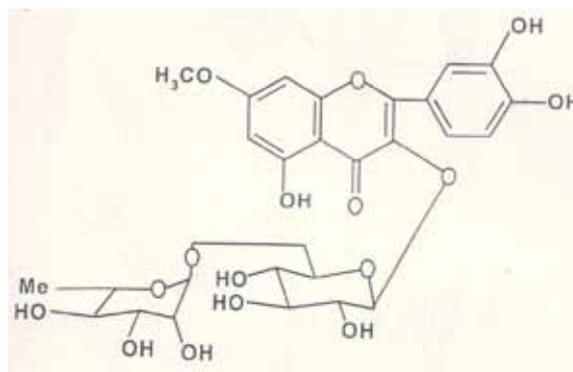


Table 1.1

Rf (x100)values of the G1 from the yellow flowers of *opuntia stricta*

(whatman No.1 Ascending,30±)

Compound	Developing Solvents								
	A	B	C	D	E	F	G	H	I
Glycoside	40	42	50	58	65	50	70	62	-
Aglycone from G1 (complete hydrolysis)	-	-	04	19	39	89	80	62	-
Rhamnetin authentic	-	-	04	19	40	90	80	62	-
Glycoside G1 (Partial Hydrolysis)	2	6	20	30	57	59	76	52	-
Rhamnetin3-O-glucoside	2	6	20	30	57	59	76	52	-

Fig.1. Rhamnetin-3-O-rutinoside

*Solvent Keys

A = H₂O, B = 5%aq.HOAc, C = 15%aq.HOAc, D=30%aq.HOAc, E=60%aq.HOAc,
 F=n.BuOH;H₂O=4:1:5(Upper phase), G=Phenol Saturated with water
 H = H O A c : c o n . H C L : H 2 O = 3 0 : 3 : 1 0 , I = t .
 BuOH:HOAc:H₂O=3:1:1.
 G1-Rhamnetin-3-O-rutinoside.

Table 1.2

R_f (X100) values of the sugar from the G1 from the flower of *Opuntia Stricta* (Whatman No:1, Ascending, 30+2)

Compound	Developing solvent				
	F	G	H	I	J
Sugar from G1	18	38	37	-	25
Glucose authentic	17	38	37	-	24
Sugar from G1	34	58	58	55	-
Rhamnose authentic	34	58	59	55	-

J=nBuOH: Benzene: Pyridine: H₂O=5:1:3:3

Spray reagent : Aniline hydrogen phthalate.

G1-Rhamnetin-3-O-rutinoside

Table 1.3

¹³C- NMR spectral data and their assignment for the aglycone and glycoside G1 from the yellow flowers of *Opuntia Stricta*

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Rhamnetin (from Literature (δ) ppm)	147.9	136	175.9	160.4	97.4	164.9	91.9	156.0	103.7
Glycoside G1 (δ ppm)	156.80	133.60	177.67	161.53	99.05	164.46	94.13	156.80	104.35
Rhamnetin 3-O-rutinoside from literature (δ ppm)	156.2	133.16	177.4	160.8	97.8	165	92.1	156.2	104.7

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	Cof – OCH ₃
Rhamnetin (from Literature (δ) ppm)	121.9	115.2	45.0	147.8	115.6	120.1	55.9
Glycoside G1 (δ ppm)	122.61	115.57	145.07	148.73	115.57	121.38	56
Rhamnetin 3-O-rutinoside from literature (δ ppm)	121.9	115.1	144.7	148.5	116.1	121.9	56.1

Compound	C-1"	C-2"	C-3"	C-4"	C-5"	C-6"
Glycoside G1 δ ppm	101.22	74.4	76.26	76.7	70.43	67.16
Glucose from literature δ ppm	101.4	74.2	76.1	76.3	70.4	67.1

Compound	C-1"	C-2"	C-3"	C-4"	C-5"	C-6"
Glycoside G1 δ ppm	100.7	74.61	72.12	70.92	68.61	18.03
Rhamnose from literature δ ppm	100.5	74.3	72.1	70.8	68.2	17.3

3. Antioxidant activity of Rhamnetin-3-O-rutinoside

3.1 Oxidative stress (OS) is a state of imbalance between generation of reactive oxygen species (ROS) like hydroxyl and superoxide radicals and the levels of antioxidant defence system³. The consequences of OS involve damage of biomolecules and abnormality in calcium metabolism, destruction of thiol group containing enzymes and inactivation of membrane-bound receptors^{4,5}. OS has been linked to cancer, anti inflammation and neurodegenerative diseases. (Parkinson's and Alzheimer's)^{6,7}.
 3.2; The present study deals with lipid peroxidation by a drug 'Ceftizoxime sodium (CZX) a third generation Cephalosporin antibiotic and in vitro evaluation of the flavonoidal drug Rhamnetin-3-O-rutinoside which component of antioxidant as inhibitors of drug induced lipid peroxidation.

3.3 Experiments;

Whole blood from goat (*Capra capra*) was collected with Al-sevier's solution as anticoagulant. The blood was centrifuged to separate the plasma from the RBC. The plasma free RBC was made into 10% suspension with isotonic saline and used for the evaluation of the antiperoxidative effect of Rhamnetin-3-O-rutinoside. The RBC suspension with CZX solution serves as control. RBC suspension and CZX was treated with the drugs at time period namely 3,6,8 & 24 hrs. The lipid peroxidation product malondialdehyde measured following thiobarbituric method of Okhawa et al 1976 8. The prepared reaction mixture was centrifuged at 3000 rpm for 15 minutes and the supernatant was used for the assay of MDA content. To this 1.5 ml TBA was added and kept in a water bath for 30 minutes and cooled. The colour developed was read at 530nm against a reagent blank.

Table 1.4. Antioxidant activity of Rhamnetin-3-O-rutinoside (G1) based on anti lipid peroxidation activity.

S.No	Drug	Lipid Peroxide level			
		50µg		100µg	
		Release (in %)	Inhibition (in %)	Release (in %)	Inhibition (in %)
1	Control	100	-	100	-
2	G1	70.55	29.44	67.48	32.52

1	Control	100	-	100	-
2	G1	70.55	29.44	67.48	32.52

4. Results and Discussion:

The antioxidant property of the Rhamnetin-3-O-rutinoside has been tabulated in Table 1.4 to study the effect on the inhibition of malondialdehyde production in the whole blood (Goat) 50 µg and 100 µg of the respective drug has been used, so the present study indicates the effect of Rhamnetin-3-O-rutinoside. On the release and inhibition of LPO level was found to be 29.44% at 50 µg and 32.52% at 100 µg level. The decrease in the levels of lipid peroxide observed in flavonoid treatment of hyperlipidemic rats suggests that these compounds can counteract the deleterious effects of a cholesterol diet.

5. Conclusion

The flowers of *Opuntia stricta* were found to contain Rhamnetin and its glycoside Rhamnetin-3-O-rutinoside. The structures of the compounds have been ascertained by chemical reactions paper chromatographic and spectroscopic techniques. The results obtained here indicate that Rhamnetin-3-O-rutinoside can act as a strong antioxidant drug.

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