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Antioxidant Activity of Patulitrin from Tagetes Patula

KEYWORDS: *Tagetespatula*, Patulitrin , Antioxidant, DPPH radical-scavenging activity.

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ABSTRACT *The fresh flowers of Tagetespatula are a shrub belonging to Asteraceae family has been found to contain Patulitrin. The structure of the above compound have been ascertained by paper chromatography, UV, 1H NMR and 13C NMR*

Spectral values. The glycoside isolated from the flowers of Tagetespatula has antioxidant property these components were used as inhibitors of the drug induced DPPH radical-scavenging activity.

INTRODUCTION:

Tagetespatula is a bushy annual plant and native of Mexico. It is cultivated in gardens all over India. It grows up to an altitude of1; 350m.The herb is grown on a comparatively large scale in Western India. The flowers have been distilled for the production of attars. The flowers contain patulitrin and its glycoside. It is used as an antiseptic fly repellant and a modifier in hair lotion. A new acyclic monoterpene glycoside, 2-methyl-6-methylene-2; 7-octadiene-1-O-β-D-glyco-pyranoside and *patulitrin* were isolated from the *Tagetespatula* flowers.

EXPERIMENTAL METHODS

Extraction and fractionation:

Fresh flowers of *Tagetespatula*(1kg) collected during December from the KodaikanalHills, in tamilnadu were extracted with 80% MeOH under reflux. The alcoholic concentrate was successively fractionated with benzene (3 x 250 ml), peroxide free Et_2O (3 X 250 ml) and EtOAc (4 X 250 ml).The benzene fraction did not yield any isolable material.

Et_2O fraction: - (Flavonol-patuletin)

The residue from the Et_2O fraction was taken up in Me_2CO and left in an ice-chest for about a week, when yellow needles (MeOH) m.p.262-264°C.It appeared yellow under UV, with or without ammonia. It responded to Horhammer-Hansel,Wilson's boricacid,Gibb's tests. It never answered Molisch's tests. It gave yellow colour with.NaOH,intense yellow with concentrated sulphuric acid and gave yellow colour with Na_2CO_3. It had λ_{max}^{MeOH} nm 258,272sh,293sh,371;+NaOMe 251sh,296sh,336,411sh;+$AlCl_3$238,275,308sh,327sh,459;+$AlCl_3$/HCl 240,268,302sh,381sh,427;+NaOAc 258sh,274sh, 340,394sh ; +NaOAc/H_3BO_3 264 , 393. It had R_f values as depicted in Table I-I. It was identified as patuletin,by direct comparison with an authentic sample.

EtOAc fraction: (Flavonal glucoside - patulitrin)

The EtOAc fraction was concentrated *in-vacuuo* and left in an ice-chest for a few days when a yellow solid separated which was filtered and studied. When crystallized from MeOH, It came out as yellow needles, m.p. 198-200°C. It was freely soluble in aqueous NaOH, hot water, EtOH and EtOAc, but insoluble in Et_2O and $CHCl_3$. It gave greenish brown colour with alc. Fe^{3+}, an intense yellow colour with NaOH, red colour with Mg-HCl and yellow precipitate with aq. Lead acetate. It appeared dull yellow under UV with or without ammonia. It answered Wilson's boric acid Horhammer-Hansel,Gibb's and Molisch's tests. It had λ_{max}^{MeOH}nm 259,273sh,388sh,373;+NaOMe 242, 292, 382, 467(dec) ;+$AlCl_3$276, 349, 462 ; +$AlCl_3$/HCl 269,302sh ,380sh ,431; +NaOAc 258,343,397sh,417sh (dec.); +NaOAc/H_3BO_3 265 , 394.It had R_f values as depicted in Table I-1. It was identified as patulitrin by observing its ^{1}H-NMR and ^{13} N-NMR values.

Hydrolysis of the glycoside:

The glycoside was dissolved in hot MeOH (0.05M) and an equal volume of H_2SO_4 was added to it. The reaction mixture was refluxed at 100 °C for 2 hrs. The excess of alcohol was distilled off in-*vacuuo* and the resulting aqueous solution was extracted with more water and left under chilled conditions for 2hrs. A yellow solid that separated was filtered, washed and dried .The aqueous filtrate and the filter paper was combined with the residue from the dried Et_2O extract studied for the aglycone.

Identification of the Aglycone: (Flavonol-patuletin)

The aglycone on recrystalisation from MeOH affored a yellow crystalline solid, which was identified as patuletin by colour reactions,behavior under UV and R_f Table I-1. It had the same UV spectral values mentioned under ether fraction.

Identification of Sugar: (Glucose)

The aq. after the removal of the aglycone was cautiously neutralized with $BaCO_3$and filtered .The concentrated filtrate on PC gave R_f values corresponding to those of glucose. The identity of the sugar was further confirmed by direct comparison with authentic sample of glucose as also by preparation of its osazone . A quantitative hydrolysis of the Folin-Wu's micro method revealed it to be a monoxide. It had R_f values as depicted in Table I-2.

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TABLE I-1
R_f(x 100) VALUES OF THE CONSTITUTENTS OF THE FLOWERS OF *TAGETESPATULA*
(Whatsmann No.1; Ascending 30 2±°C)

Compound	Developing solvents*								
	A	B	C	D	E	F	G	H	I
Aglycone from Et_2O fraction		2	4	10	25	68	56	54	48
Patuletin (authentic)		2	4	10	25	68	56	54	48
Flavones glycoside from Et_2OAc fraction	10	5	15	30	62	75	60	15	67
Patulitrin (authentic)	10	5	15	30	62	75	60	15	67

*Solvent key

A-H2O
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 (X 100) VALUES OF THE SUGAR FROM THE GLYCOSIDE FROM *TAGETES PATULA*
(Whatsman No.1; Ascending 30 ±2°C)

Compound	Developing solvent			
	E	F	G	H
Sugar from the hydrolysate of EtOAc fraction	77	09	39	90
Glucose (authentic)	77	09	39	90

TABLE I-3
^{13}C – NMR DATA AND THEIR ASSIGNMENT FOR THE GLYCOSIDE FROM *THE FLOWERS OF TAGETES PATULA*

Compound	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Glycoside (dppm)	147.7	135.5	176.1	151.5	129.6	159.2	93.6	151.5	105.0
Compound	C_1'	C_2'	C_3'	C_4'	C_5'	C_6'			
Glycoside (dppm)	122.0	115.3	145.0	147.4	115.5	119.9			
Compound	C_1''	C_2''	C_3''	C_4''	C_5''	C_6''			
Glycoside (dppm)	73.1	69.6	77.2	69.6	77.2	60.6			

ANTI OXIDANT ACTIVITY
MATERIALS AND METHODS
Preparation of extract
Different concentrations of plant extract (20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992).

Reagents;
1. DPPH : 25 µg/ml in methanol
2. Methanol

Procedure:
Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{Ac - AS}{Ac} \times 100 \right)$$

Where $A_{C=}$ control is the absorbance and $A_{S=}$ sample is the absorbance of reaction mixture (in the presence of sample).

Tagetespatula

Table DPPH radical scavenging activity

S.NO	Concentrations (µg/ml)	*Tagetespatula*	Ascorbic acid (Standard)
1	20	18.18±1.27	25.6±2.04
2	40	36.31±2.54	61.26±4.90
3	60	49.95±3.49	88.98±7.11
4	80	72.5±5.07	99.34±7.94
	IC_{50}	56.52	35.03

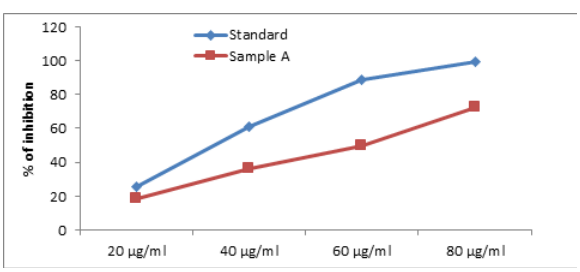

Fig DPPH radical scavenging activity

RESULT AND DISCUSSION
Tagetespatula is screened for its antioxidant activity L-Ascorbic acid is used as standard. its determined by the method of shimada,et al.,(1992).The antioxidant activity of *Tagetespatula* is given in the above table .The drug solution is prepared according various concentration like 20µl,40µl,60µl and 80µl.The DPPH radical scavenging activity of *Tagetespatula* the higher concentration (80 µl) of the drug is more active than lower concentration (20 µl).At higher concentration the percentage of inhibition is more active than the lower concentration.

CONCLUSION
The flowers of *Tegetespatula* found to contain *Patulitrin*. The structure of the compounds has been ascertained by chemical reactions paper chromatographic and spectroscopic techniques. The result obtained here indicate that *Patulitrin* can act as a strong antioxidant drug.

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