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Antioxidant activity of Buddleja asiatica

KEYWORDS: Buddleja asiatica hesperitin -7-O-rutinoside-antioxident-thiobar bituric.

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ABSTRACT *The fresh flowers of buddleja asiatica how is a shrub belonging to buddlejaceae family have been found to contain hesperitin and its glycoside hesperitin -7-0 rutinoside) The structure of the above compounds have been ascertained by paper Chromatograph, UV, 13C NMR and 1H NMR spectural values. The Glycoside isolated from the flower of Buddlejacea asiatica has antioxidant property these components were used as inhibitors of drug induced lipid peroxidation.*

INTRODUCTION

Buddleja asiatica lour. belongs to the family Buddlejaceae and is native of Eastern Asia. Leaves, seeds and roots are purgative. The seeds are used externally for skin diseases. The dried plant is also used as fuel. Young leaves are crushed with a piece of turmeric which is applied externally to cure scabies. Field surveys showed that B.asiatica has an extraordinary accumulation capacity and tolerance for Pb4.

The alcoholic extract obtained from the leaves of this plant has been observed to produce a persistent and prolonged fall of blood pressure in pentobarbitone-anaesthetised dogs and cats. From the flowering parts of B.asiatica, steroids (beta-sitosterol, stignasterol, stigmasterol-O-glucoside, beta-sitosterol-O-glucoside), iridoid glucoside (methyl catalpol, acucbin), phenylpropanoids (isoacteoside and acteoside), triterpene saponin (mimengoside A), flavonoids (diosmin and linarin) in addition to the free sugars mannitol and sucrose have been reported.

EXPERINMENTAL METHODS

Extraction and fractionation:

Fresh flowers (1 kg) of B.asiatica collected from in and around Kumbakonam during June were extracted with 85% EtOH (4 x 500 ml) under relux. The alc.extract was concentrated in vacuo and the aq.concentrate successively fractionated with beneze (3 x 250ml), peroxide-free Et2O (3 x 250 ml) and EtOAC (4 x 250 ml).

No crystalline solid could be recovered from benzene and Et2O fractions.

EtOAc fraction : (Flavonone glycosisde: Hesperitin)

The EtOAc fraction was concentrated in vacuo and

left in an ice chest for a few days. A colourless solid that separated was filtered and studied. It developed a crimson red colour with Mg-HCl. It appeared pale yellow under UV. It responded to Wilson's boric acid, Molisch's and Gibb's tests but did not answer the Horhammer –Hansel test. It had λ_{max}^{MeOH} nm 283, ; (+NaOMe) 242, 286, 356; (+AlCl3) 308,383; (+AlCl3/HCl) 306, 379; (+NaOAc) 284, 328; (_NaOAc/H3BO3) 284, 326. It had Rf values as depicted in table -1 The 1H- and 13C NMR of the glycoside are appended.

Hydrolysis of the glycoside:

The glycoside (0.05g ≠ 0.2 m mole) dissolved in hot aq.MeOH (2 ml, 50%) was hydrolysed with H2SO4 (5%) at 100 °C for about 2 hr and the hydrolytic products identified as described below.

Identification of the aglycones: (flavanone :hesperitin)

The aglycones on recrystallisation from methanol gave yellow leaflets, m.p. 316 – 18 °C (yield 0.02%). A colourless solid that separated was filtered and studied. On reduction in ethanol with magnesium and hydrochloride, it gave intense red colour. It appeared pale yellow under UV. It responded to Wilson's boric acid and Gibb's tests but did not answer the Molischs' and Horhammer – Hansel tests. It hadnm226,288,330;

(+NaOMe) 223,285,365; (+AlCl3) 313, 354; (AlCl3/HCl) 310, 350; (+NaOAc) 323,352; (+NaOAc/H3BO3) 283, 329. It had Rf values as depicted in table 1.

Identification of the sugar: (glucose and rhamnose)

The aqueous hydrolysate after the removal of the aglycone was neutralized with BaCO3 and filtered. The concentrated filtrate on PC gave Rf values (Table -1) corresponding to those of glucose and rhamnose. The running properties of the glycoside were also in favour of a bioside. The identity of the sugar was also confirmed by direct comparison with authentic samples of glucose and rhamnose.

Partial hydrolysis of the glycoside:

The glycoside was subjected to partial hydrolysis by treatment with 10% formic acid in cyclohexane and the resulting solution extracted with ethyl acetate and subjected to PC. The Rf values of the EtOAc fraction agreed with those of hesperitin-7-O-rutinoside. The Rf values are indicated in Table - 1. On this basis it can be concluded that glucose is directly linked to the aglycones moiety

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TABLE - 1.

R_f (x 100) VALUES OF THE CONSTITUENTS OF THE FLOWERS OF

BUDDLEJA ASIATICA

(Whatman NO.1 Ascending, 30± 2 °)

Compound	Developing Solvents*							
	a	b	c	d	e	f	g	h
Glycoside from EtOAc fraction	50	24	41	84	90	48	62	79
Hesperitin (Authentic)	50	23	41	84	90	47	62	78
Gyloside (Partial Hydrolysis)	33	19	38	79	82	76	67	65
Hesperitin 7-O-ru (Authentic)	33	18	38	79	83	76	66	65
Aglycone from the above Glycoside	-	-	25	64	76	89	44	46
Hesperitin (Authentic)	-	-	24	63	75	89	44	45

*Solvent Key a – H_2O
b – 5% aq.HOAc,
c – 15% aq.HOAc,
d – 30% aq.HOAc,
e – 60% aq.HOAc,
f – BAW (n-BuOH: HOAc: H_2O = 4:1:5, upper phase)
g – Phenol saturated with water.
H – forestol (HOAc : Conc. HCl : H_2O = 30: 3:10)

TABLE - 2
Rf VALUE (X 100) VALUE OF THE SUGAR FROM THE G1 FROM FLOWER OF BUDDLEJA ASIATICA (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	24	58	58	55	-
Hesperitin authentic	34	58	59	55	-

J = nBuOH: Benzene : pyridine : H_2O

Spray reagent : Aniline hydrogen phthalate

G1 – Hesperitin – 7 – O – rutinoside

TABLE -3
13C – NMR SPECTRAL DATA AND THEIR ASSIGNMENT FOR THE GLYCOSIDE FROM THE FLOWER OF BUDDLEIA ASIATICA

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Hesperitin from the literature (δ ppm)	156.3	42.0	196.7	163.0	96.7	165.2	95.8	162.5	103.5
Glycoside (δ ppm)	78.3	42.0	196.9	163.0	96.4	165.1	95.5	162.5	103.6

Compound	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
Hesperitin from the literature (δ ppm)	131.2	114.3	148.1	146.7	112.7	117.8
Glycoside (δ ppm)	130.9	114.1	147.9	146.4	112.1	117.9

Compound	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
Hesperitin from the literature (δ ppm)	99.8	73.3	76.6	69.9	75.5	66.4
Glycoside (δ ppm)	99.5	73.0	76.3	69.9	75.5	66.0

Compound	C-1‴	C-2‴	C-3‴	C-4‴	C-5‴	C-6‴
Hesperitin from the literature (δ ppm)	100.7	70.6	71.0	72.4	68.6	18.2
Glycoside (δ ppm)	100.6	70.2	70.7	72.1	68.3	17.8

Antioxidant activity of Hesperitin -7 – O - rutinoside.
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 system-3. 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 Alzbeimer'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 Hesperitin -7 –O – rutinoside which component of antioxidant as inhibitors of drug induced lipid peroxidation.

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 Hesperitin -7 – O – rutinoside. The RBC spension 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 MAD 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 4
Antioxidant activity of Hesperitin -7 – O – rutinoside (G1) based on anti lipid peroxidation activity.

S.NO	Drug	Lipid Peroxide level			
		50 µg		100 µg	
		Release (in%)	Inhibition (in%)	Release (in%)	Inhibition (in%)
1	Control	100	-	100	-
2	G1	71.21	29.14	67.17	31.52

4.Results and Discussion:
The antioxidant property of the Hesperitin -7 – 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 and 100µg of the respective drug has been used. So the present study indicates the effect of Hesperitin -7 – O – rutinoside. On the release and in-

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hibition of LPO level was found to be 29.14% at 50µg and 31.52% at 100µg 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 cholesterol diet.

Conclusion

The flowers of Buddeja asiaficawer found to contain Hesperitin and Hesperitin its glycoside -7 – O – rutinoside. The structures of the compounds have been ascertained by chemical reactions paper chromatographic and spectroscopic techniques. The results obtained here indicate that Hesperitin -7 – O – rutinoside can act as a strong antioxidant drug.

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