(110 mg), m.p. 209–10° (Found: C, 57.20; H, 4.60. Calc. for $C_{22}H_{20}O_{11}$: C, 57.39; H, 4.34%). It gave yellow colour with alkali, pink colour with Mg-HCl, brown colour with Fe^{3+} and positive Molisch's test suggesting that it could be a flavonoid glycoside. It had $[\alpha]_D^{28} = -50°$ (c, 1.0. C_5H_5N); UV (λ_{max}): 275, 340 (MeOH), 280, 330 sh, 348, 390 sh ($AlCl_3$), 275, 340 (NaOAc), 275, 340 (NaOAc/H_3BO_3) and 280, 388 nm (NaOMe); IR: 3400 (br), 2925, 1720, 1640, 1600, 1480, 1250, 1100 (br), 1040, 940, 860, 780 and 695 cm^{-1}; PMR (90 MHz, DMSO-d_6): δ 11.4 (br s, 2H, COOH and 5-OH), 7.4–8.2, centred at 8.0 and 7.6 (pair of m, 5H, H–2′, 3′, 4′, 5′ and 6′), 6.9 (s, 1H, 3–H), 6.66(s, 1H, 6–H), 4.0 (s, 3H, OCH_3) and 3.6 (br s, H of sugar/water).

The glycoside showed high R_f in water, with marked decrease in 5% HOAc (typical of glucuronides)[8]. It resisted mild acid hydrolysis (1 N HCl, 1 hr). On refluxing with 2 N HCl for 3 hr, as well as when treated with the enzyme β-glucuronidase, it underwent hydrolysis and yielded an aglycone and D-glucuronic acid in equimolar ratio. The aglycone was identified as wogonin.

The glycoside on acetylation ($Ac_2O + C_5H_5N$, 28°, 24 hr) gave the lactone acetate, C_{28} H_{24} O_{13}, m.p. 189–90°, $[\alpha]_D^{28} = -96°$ ($CHCl_3$), PMR (90 MHz, $CDCl_3$): δ 7.3–8.0, centred at 7.9 and 7.5 (pair of m, 5H, H, 2′, 3′, 4′, 5′ and 6′), 6.9 (s, 1H, 3–H), 6.6 (s, 1H, 6–H), 5.74 (s, 1H, 1″–H), 5.52 (d, J = 4Hz, 1H, 2″–H), 5.0 (t, J = 4Hz, 1H, 4″–H) 4.4 (d, J = 4Hz, 1H, 5″–H), 4.02 (s, 3H, 8–OCH_3), 2.44 (s, 3H, 5–$OCOCH_3$), 2.24 and 2.18 (s, each, 3H each, 4″ and 2″–$OCOCH_3$); MS: (m/z) 526 (lactone acetate–$CH_2 = CO$), 326 (5-acetyl aglycone), 284 (aglycone), 269 (284–15), 255, 243, 167 (A-ring), 139 (167–28), 111 and 97.

Identical λ_{max} of the glycoside and its aglycone, as also the absence of any shift in the NaOAc spectrum of the glycoside, established the involvement of 7–OH in glycosylation[4,9]. The enzyme hydrolysis showed that the sugar was β-linked, while the PMR spectrum revealed its pyranoside structure. Thus the flavonoid has been characterized as 5-hydroxy-8-methoxy-7-O-β-D-glucopyranuronosylflavone (oroxindin). The identity was unequivocally established by direct comparison (m.m.p., co-TLC, IR) with an authentic sample of oroxindin obtained from *Oroxylum indicum*[10].

Oroxindin has been isolated for the first time from a plant belonging to the family Verbenaceae. Its earlier occurrence has been reported only in two other plants, *Oroxylum indicum*[10] (Bignoniaceae) and *Scutellaria galericulata*[11] (Labiatae). It is interesting to note that the flavonoid wogonin, devoid of any B-ring oxygenation (possessing unsubstituted B-ring) occurs along with its glycoside, oroxindin in *H. sanguinea*, a member of the Verbenaceae in the Tubiflorae, as unsubstituted B-ring flavones have been reported mostly in the Tubiflorae[4,11].

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INHIBITION OF ASCORBATE AUTOXIDATION BY HUMAN BLOOD

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HUMAN and rat blood sera have been shown to contain powerful protective factors against *in vitro* lipid auto-

xidation[1,2]. Further, blood plasma has been reported to inhibit the autoxidation of epinephrin[3] DOPA[4] (3,4-dihydroxyphenylalanine) and bilirubin[5]. Recently Mishra and Kovachich[6] demonstrated that mammalian nervous tissues and serum contain a high molecular weight substance that protects reduced ascorbic acid against *in vitro* autoxidation. The present study reports the presence of a factor in human blood, erythrocytes and plasma that protects *in vitro* oxidative autoxidation of ascorbic acid, on incubation in air. Blood was collected from healthy human volunteers by venous puncture into heparinized syringes; the plasma and the erythrocytes were separated by centrifugation. The erythrocyte stroma was prepared according to Dodge *et al*[7] with slight modifications. The washed cells were lysed by mixing them with 3 volumes of cold distilled water and centrifuged at 4°C at 20,000 g for 20 min. The ghost pellet was repeatedly washed with the same volume of distilled water as above, till the supernatant became clear. The membrane pellet was finally suspended as a fine dispersion in cold salinephosphate buffer (pH 7.4) in the required dilution.

Ascorbic acid autoxidation was assayed following the procedure described earlier[6]. The assay mixture contained 0.2 mg L-ascorbic acid (Merck and Co) in salinephosphate buffer, 0.005 M, pH 7.4 and 0.2 ml of diluted plasma in a final volume of 10 ml. The reaction mixture was incubated at 37°C in uncorked test tubes in a water bath; two ml portions were pipetted out at given time intervals, and the absorbancy at 265 nm was measured directly using a spectrophotometer, for determining the unoxidized ascorbic acid in the sample.

In duplicate experiments similar aliquots were used for titration against 2,6 dichlophenol indophenol, for measuring the concentration of the reduced ascorbic acid at different time intervals of incubation; and the total ascorbic acid (reduced and dehydro) content was also measured in the samples using the method of Roe and Kuether[8]. Wherever dilutions were necessary for estimation, double-distilled water was used. Protein was estimated by the method of Reinhold[9] using crystalline bovine albumin as the standard.

Table 1 summarizes the effects of different dilutions of normal human plasma on the autoxidation of ascorbic acid. Ascorbic acid on incubation in air is found to steadily undergo autoxidation and at the end of 3 hr incubation only 19% was recovered as unoxidized ascorbic acid. Normal human plasma showed a marked antioxidant property on ascorbite autoxidation as may be seen from the results in table 1; even with plasma at 10^4 dilution (equivalent to 70 μg proteins, i.e. 1 μl of plasma in the reaction mixture) inhibited autoxidation at all the time intervals studied. Higher concentrations of plasma (5×10^3 to 5×10^2 dilutions) afforded higher protection against ascorbate oxidation though not in strict proportion to the plasma concentration. The above results were confirmed by absorbancy measurement at 265 nm, which is reported to be specific for reduced ascorbic acid. The above findings were also confirmed by titration of aliquots with 2,6 dichlophenol indophenol and estimation of total ascorbic acid[8].

In table 2 is presented the antioxidant activity against ascorbate autoxidation of the whole blood, erythrocytes, plasma and RBC stroma, at 1 in 5,000 dilutions. The results show that the whole blood

Table 1 *Effect of normal plasma on autoxidation rate of ascorbic acid at plasma dilutions ranging from* 5×10^2 *to* 1×10^4

Plasma dilution factor	Percentage of ascorbic acid remaining in the reduced form		
	1 hr	2 hr	3 hr
5×10^2	89.1 ± 1.1	78.9 ± 2.0	66.4 ± 2.5
0.667×10^3	89.1 ± 1.0	78.9 ± 2.5	65.7 ± 2.5
10^3	88.1 ± 1.5	76.8 ± 2.7	65.7 ± 2.8
2×10^3	85.7 ± 1.4	76.7 ± 2.9	65.0 ± 3.0
2.5×10^3	85.6 ± 1.3	76.1 ± 2.9	56.5 ± 3.2
3.33×10^3	85.1 ± 1.2	74.4 ± 2.8	55.8 ± 3.1
5×10^3	83.2 ± 1.2	73.3 ± 3.0	55.7 ± 3.2
1×10^4	82.8 ± 1.3	66.9 ± 3.5	39.4 ± 4.0
Control (without plasma)	68.8 ± 5.1	40.7 ± 6.8	19.6 ± 8.0

Results are the mean of ± SD of 3 different experiments.

Table 2 *Autoxidation rate of ascorbic acid as measured by absorbancy at 265 nm in the presence of inhibitory factors like normal human whole blood, red blood cells, plasma and RBC stroma.*

Additions	Percentage of ascorbic acid remaining unoxidised		
	1 hr	2 hr	3 hr
Whole blood	92.4 ± 0.5	84.4 ± 0.7	80.3 ± 0.8
Red blood cells	88·5 ± 0.5	81.9 ± 0.8	67.2 ± 1.2
Plasma	83.2 ± 1.2	73.3 ± 3.0	55.7 ± 3.2
RBC stroma	55·6 ± 4.5	31.0 ± 5.1	6.9 ± 6.0
Control	68.8 ± 5.1	40.7 ± 6.8	19.6 ± 8.0

Results are the mean of ± SD of 3 different experiments.

exhibited higher antioxidant activity than either the component erythrocytes or plasma.

It is quite interesting that RBC stroma increased autoxidation rate significantly.

These results confirm the earlier reports[6] which showed that soluble fractions of mammalian tissue and serum show significant inhibitory activity towards autoxidation of L-ascorbic acid. Human serum has been shown to contain powerful antioxidant activity towards unsaturated lipids[1,2], catecholamines[3] and bilirubin[5] and the protective component of the human serum against lipid autoxidation has been identified[10] as ceruloplasmin. However, our unpublished observations indicate that both Cu^{++} ions and ceruloplasmin enhanced the ascorbic acid autoxidation. The nature of the protective factor, in blood, is therefore still not clear and work is in progress to identify this factor.

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A SCREENING MEDIUM FOR L-ASPARAGINASE PRODUCTION BY *ERWINIA CAROTOVORA* AND *PSEUDOMONAS PUTIDA*

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BROOM[1] presented evidence to show that the enzyme L-asparaginase present in guinea pig serum was responsible for the antitumor properties which could suppress some transplanted lymphomas of mice and rats. Subsequent findings[2] indicated that the antitumor activity of L-asparaginase obtained from *E. coli* was quite similar to guinea pig serum. This opened up the possibility of large scale microbial production of the enzyme for clinical trials. An attempt was made to develop a reasonably simple medium with minimal amounts of yeast extract and tryptone (the basis being glucose and asparagine) which proved very useful for screening the two genera *Pseudomonas* and *Erwinia*. The latter genus was chosen since it produced tumor active enzyme and the former as a control.

Cultures: Fiftytwo strains of *E. carotovora* and 46 strains of *P. putida* were used in this study. The former were isolated from plants and the latter from soil. All these strains were tested for L-asparaginase activity.

Medium and cultivation conditions: The medium developed was a simple salt solution that contained (g/l); $HO_2CCH_2C(OH)(CO_2H)\ CH_2CO_2Na$, 0.5; $MgSO_4.7H_2O$, 1.0; NaCl, 1.0; glucose, 5.0; K_2HPO_4, 0.7; KH_2PO_4, 1.05; NH_4NO_3, 0.5; yeast extract, 0.5; tryptone 0.5; L-asparagine 5.0; The final pH was adjusted to 5.53–5.56. The medium (80 ml) was distributed in 500 ml flasks and sterilized at 115°C for 20 min. The flasks were inoculated with Tryptone glucose yeast extract agar (TGY agar) cultures of the strains grown for 18–20 hr at 28°C and incubated at 37°C with shaking (220–230 rpm) for 16–46 hr. The enzyme activity was determined after harvesting the flasks according to the method of Wriston[3]. Results of activity were calculated to International Units (I.u.).

Results and discussion

Out of the 46 strains of *P. putida* tested, only one strain NBPP-18 showed activity for L-asparaginase upto 2.00 I.u/ml. The final pH was 8.8 after 42 hr of fermentation (table 1). The minimum and maximum