ANTI-INFLAMMATORY ACTIVITY OF *JUSSIEUA REPENS* LINN

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JUSSIEUA REPENS Linn belonging to Onagraceae is an aquatic weed occurring in tropical countries. The phytochemical and pharmacological studies of this plant have not yet been reported. We report here the presence of rutin and the anti-inflamatory activity of the flower petals of *J. repens*.

Fresh petals (1 kg) were collected from the outskirts of Madurai and soxhlet extracted with 50% MeOH and fractionated[1]. By preparative PC (Whatman No. 3, ascending, 15% HOAc), a flavonoidal glycoside was isolated. It was confirmed to be Quercetin 3-O-rhamnoglucoside by m.m.p., λ_{max}, characteristic shifts with diagnostic reagents[2], R_f, acid hydrolysis and direct comparison with authentic sample.

The method of Ignatius *et al*[3] was adopted to study the inhibition of carrageenin-induced rat paw oedema. After removal of MeOH from the 50% methanolic extract the resulting crude aqueous concentrate of *J. repens* was injected i.p. at doses of 100, 150 and 200 mg/kg b.w. to three different groups of rats (5 in each group). Hydrocortizone (150 mg/kg b.w.i.p.) was employed as the standard drug. The results were expressed as increase in foot volume in ml over the initial volume (table 1).

A dose-dependent inhibition of carrageenin-induced rat paw oedema comparable to that of hydrocortizone was observed. It is well known that rutin and related compounds have remarkable anti-inflammatory activity[4]. These reports indicate that the anti-inflammatory activity of *J. repens* may be due to the presence of Quercetin 3-O-rhamnoglucoside isolated from the aqueous concentrate.

Table 1 *Effect of hydrocortizone and J. repens on carrageenin-induced rat paw oedema*

Treatment	Dose mg/kg bw	Mean increase in paw volume (ml)	% inhibition
Control	—	0.62	—
Hydrocortizone	150	0.28	55
J. repens	100	0.42	32
	150	0.37	40
	200	0.33	46

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A SIMPLE TURBIDOMETRIC ASSAY FOR CONCANAVALIN A

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CONCANAVALIN A (ConA) has found a variety of applications in biochemistry[1,2]. Thus a simple and reliable assay for estimating its biological activity is fairly useful. The existing quantitative assay for

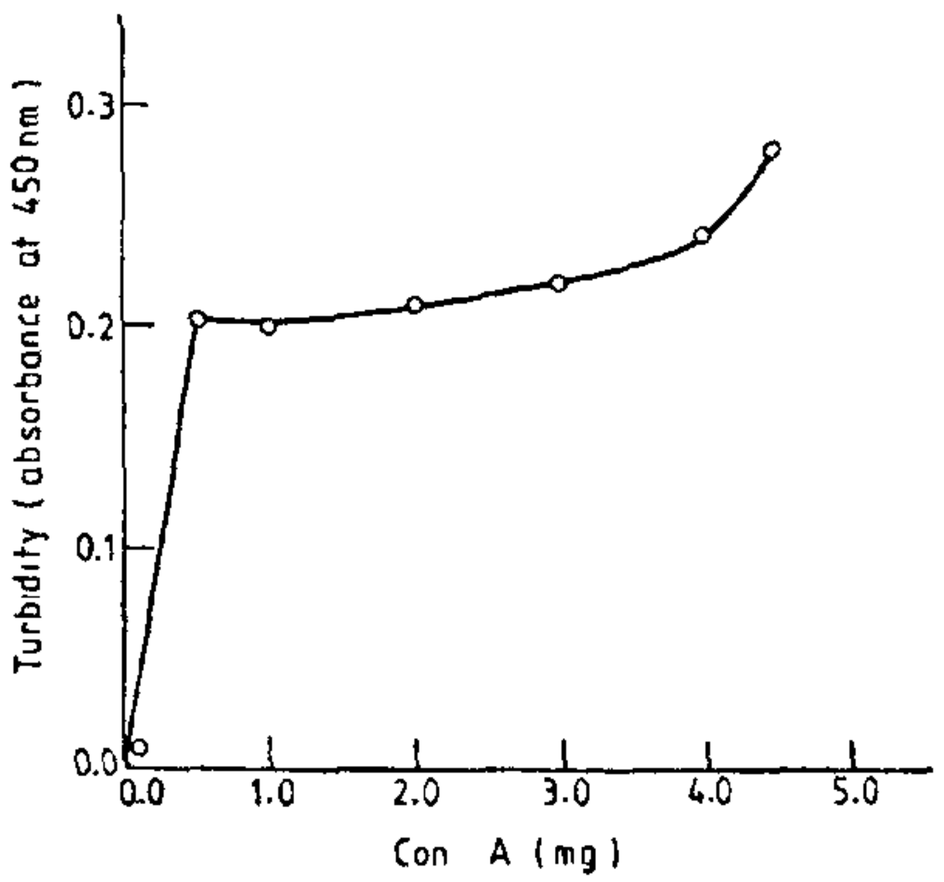

Figure 1. Turbidity as a function of ConA concentration. Stock solutions of glycogen containing 0.25 mg per ml of acetate buffer (0.05 M, pH 5.5) and ConA containing 5 mg/ml of the same buffer were prepared. Varying amounts of ConA solutions containing desired amounts of ConA were added to 1 ml of glycogen solution. Final volumes in each case were made up to 3 ml by adding appropriate volumes of the acetate buffer. Incubation was carried out at room temperature for 10 min and absorbance was measured at 450 nm.

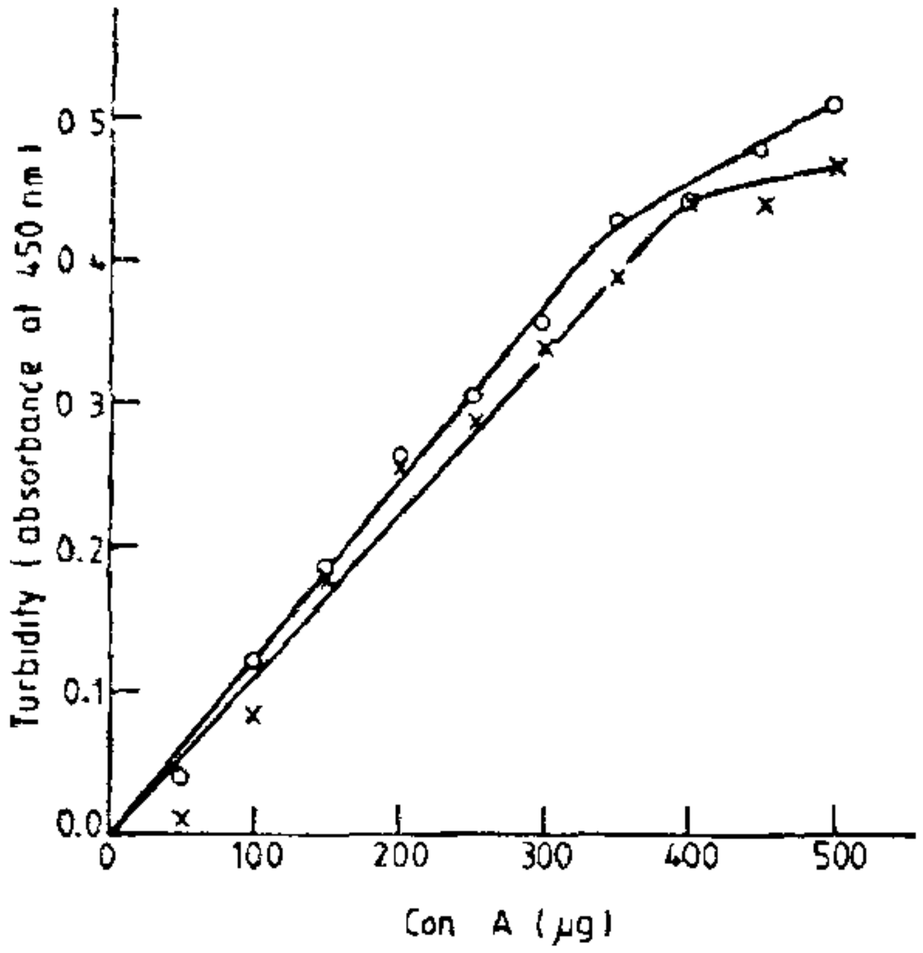

Figure 2. Turbidity as a function of ConA concentration with different time intervals. Stock solutions of glycogen containing 1 mg per ml of phosphate buffer (0.2 M; pH 7) and ConA containing 1 mg per ml of the same buffer were prepared. The reaction mixture contained 1 mg glycogen and varying amounts of ConA in a final volume of 3 ml. Incubation was carried out at room temperature (22°C) for 1 hr (×); 16 hr (○) and absorbance was measured at 450 nm.

ConA is based on precipitation of dextran and is quite cumbersome[3]. The interaction of glycogen with ConA has been studied by several workers[4–6]. In this work, we describe an assay which utilizes this interaction.

ConA was bought from the Council of Scientific and Industrial Research, Centre for Biochemicals.

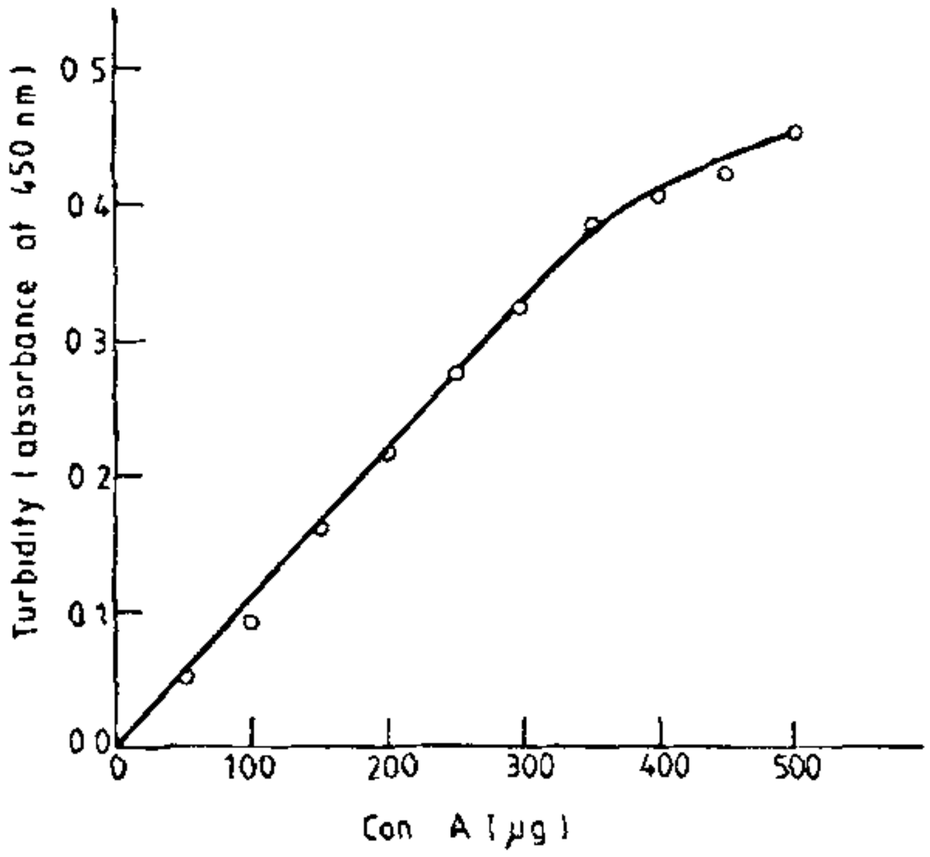

Figure 3. Turbidity as a function of ConA concentration incorporating NaCl in the assay medium. The assay buffer contained 0.33 M NaCl. Other details were the same as described for figure 2.

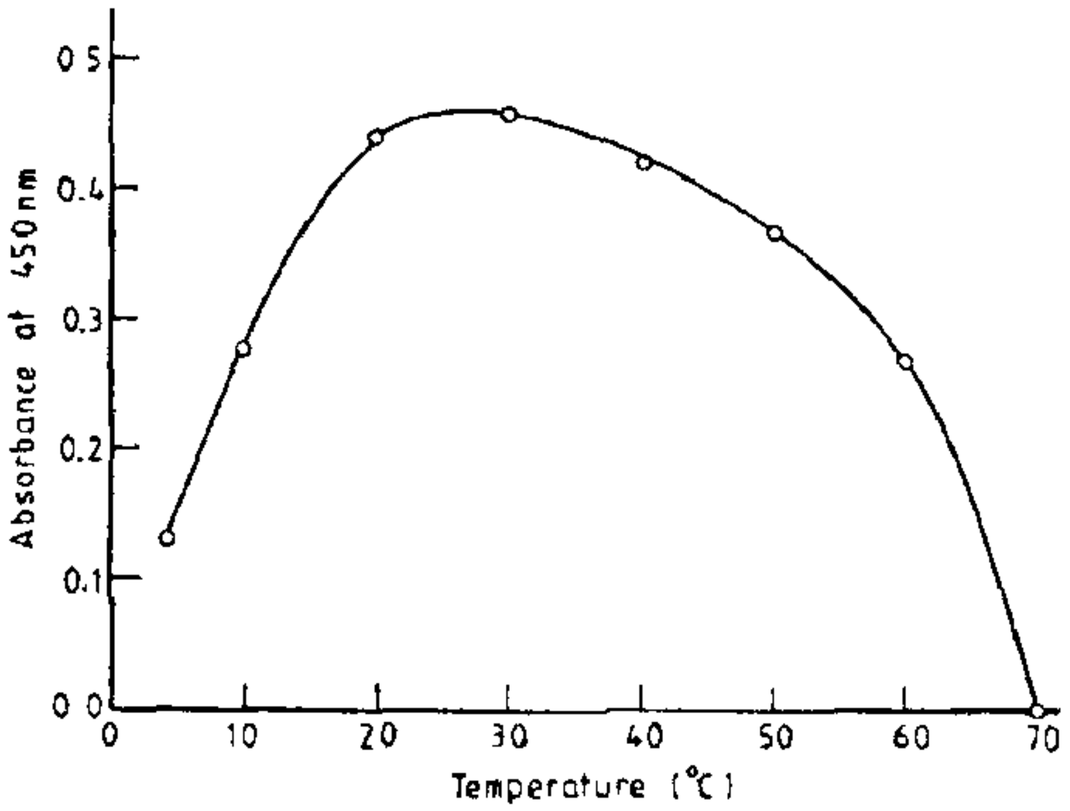

Figure 4. Turbidity as a function of temperature. The assay conditions were the same as described for figure 3.

Glycogen was obtained from E. Merck, Darmstadt (Germany). All other reagents were of analytical grade.

Like all other lectins, ConA is generally assayed by following the blood cell agglutination[7]. However, this convenient assay is at best a semiquantitative one[8]. Doyle *et al*[9] used the interaction of ConA with glycogen as a basis for estimating ConA. The

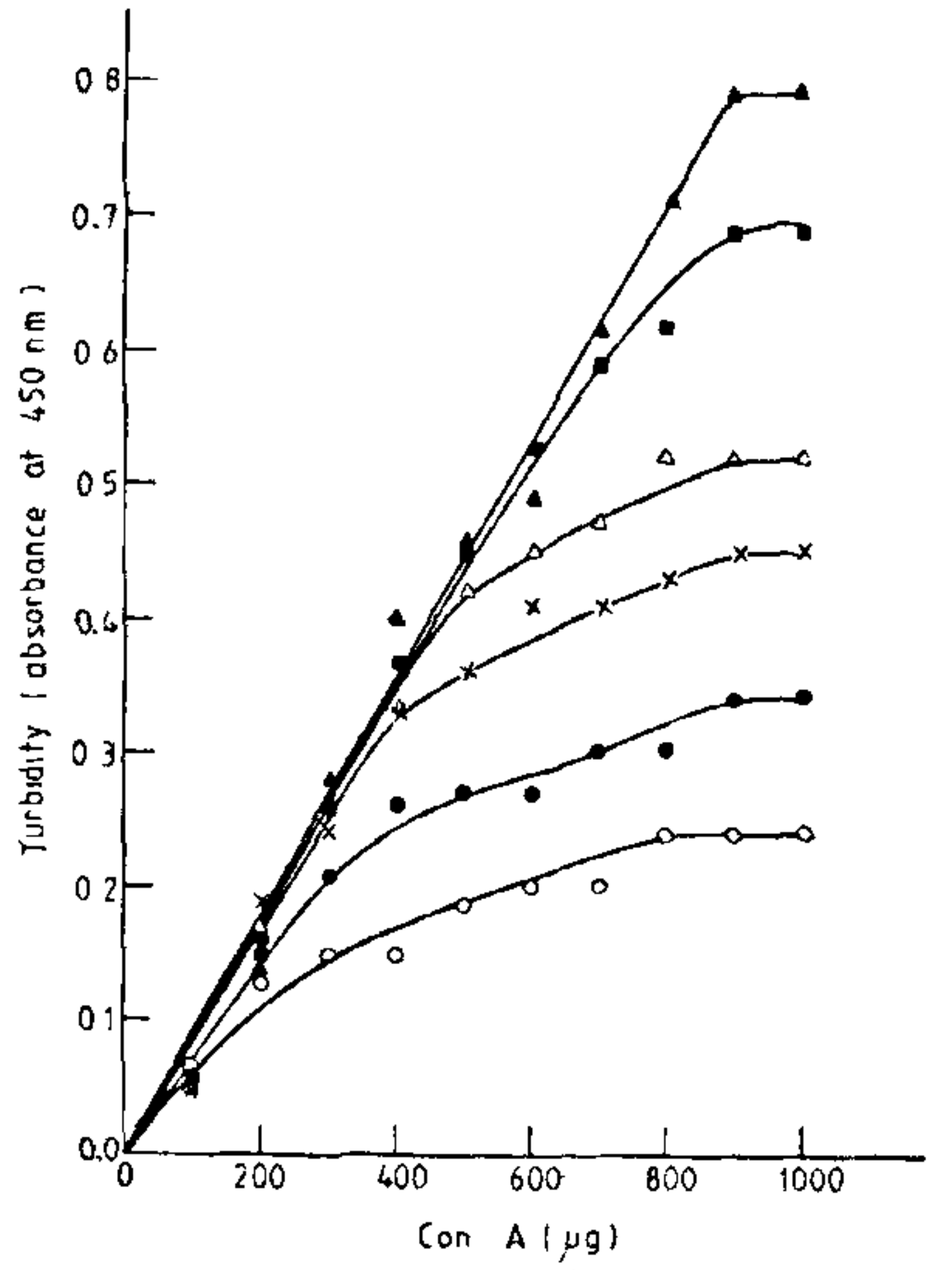

Figure 5. Turbidity as a function of ConA concentration with varying amounts of glycogen. The assay conditions were the same as described for figure 3 0.25 mg (○); 0.50 mg (●); 0.75 mg (×); 1.0 mg (△); 1.5 mg (■); 2 mg (▲).

turbidity produced on mixing glycogen with ConA was measured by them colorimetrically. Doyle *et al*[9] used 4 mg ConA in 3 ml assay buffer. When ConA concentration was varied from 0 to 5 mg, the turbidity was not proportional to ConA concentration (figure 1). Thus, it was obvious that the method could not be readily used for assaying ConA activities.

It was found that when ConA at lower concentration was incubated with 1 mg glycogen in 3 ml assay buffer, the change in turbidity values was linear with varying concentrations of ConA (figure 2). The amount of turbidity remained constant over a long period of time (i.e. 16 h). Linearity was considerably improved, particularly in the region of ConA concentration of 50–200 μg, if 0.33 M NaCl was incorporated in the assay buffer (figure 3). This was possibly due to decrease in turbidity values of ConA controls (minus glycogen) because of incorporation of the salt. The latter is known to minimize ConA precipitation in solution[10]. The influence of varying incubation temperatures on the interaction was also examined (figure 4). The results obtained are different from those reported by Doyle *et al*[6]. Unlike Doyle *et al*[6], we found that increase in temperature resulted in increase in turbidity up to 30°C. Our data, in fact, agree with that of So and Goldstein[11] who described that ConA-polysaccharide interactions are temperature-dependent and quite similar to antigen-antibody interactions. The polysaccharide used by So and Goldstein[11] was dextran. Based on the data of figure 4, any temperature between 20 and 30°C could be used for assaying ConA.

Further optimization of the assay method involved varying glycogen concentration (figure 5) and pH (figure 6). The glycogen concentration of 1 mg (as well as 1.5 mg) in the total reaction volume was found to give best results. The amount of turbidity also varied with pH and pH 7 turbidity was found to change linearly with ConA concentration up to 400 μg. Corresponding to higher concentration range of ConA (250–500 μg); pH 6.5 gave better results. We have generally used 1 mg glycogen and pH 7 for assaying ConA and this convenient assay for ConA has been found quite useful during our crosslinking studies of ConA[12].

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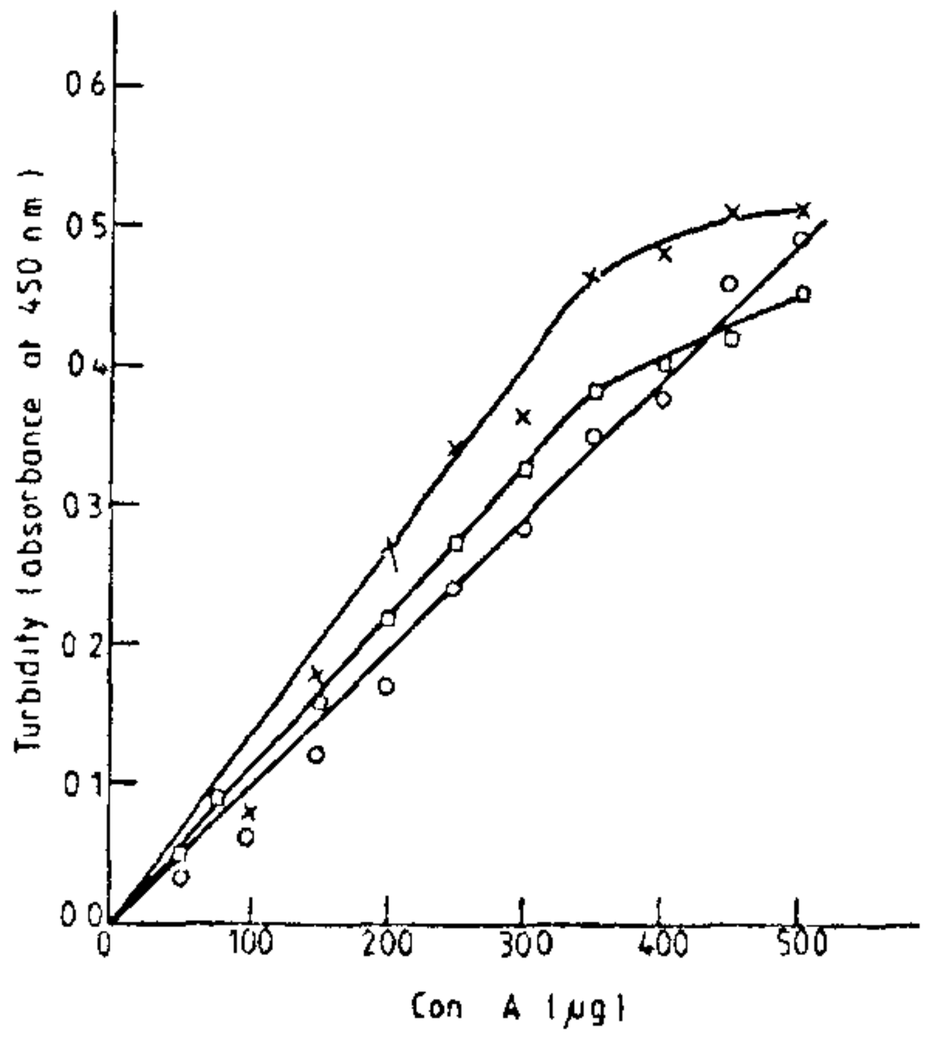

Figure 6. Turbidity as a function of ConA concentration at different pH. The assay conditions were the same as described for figure 3. pH 6.5 (○); pH 7 (□); pH 5.5 (×).

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