Enzymatic Spectrum of Herbal Plants *Plumbago* Linn.

B.N. POUL*, D.S. Mukadam† L.B. DAMA‡ and B.V. JADHAV‡
Department of Medicinal Chemistry
Maharashtra College of Pharmacy
Nilanga (Latur)-413 521, India

The family Plumbaginaceae have been surveyed for enzymatic screening in roots of the plants *Plumbago* Linn. In the present enzymatic screening all the four species of *Plumbago* were used *viz.*, *Plumbago indica*, or *Plumbago rosea*, *Plumbago zeylanica*, *Plumbago capensis* and *Plumbago albus* or *Plumbago europea* etc. The root extracts of the selected plants are used in ayurvedic digestive formulations, have shown effect of gastro-intestinal flora normaliser. The investigations were directed into the possibilities of the presence of some powerful enzyems, *viz.*, hydrolases, invertase, proteases, oxido-reductases, glucoxidase in the root power of *Plumbago* species.

The statistical data show the presence of the above enzymes in the ratio maximum 10.35 and minium 1.35 units per dry weight in *Plumbago zeylanica*, *Plumbago capensis*, *Plumbago rosea* and *Plumbago albus* respectively. The *Plumbago zeylanica* Linn. bearing white flowers shows greater effect on digestive stimulus activity, due to high concentration of enzymes, than the other *Plumbago* species, in albino mice and albino rats etc.

Chitraka (*Plumbago zeylanica*) has been known in ancient Indian medicine. The roots of Chitraka are extensively used in Indian pharmaceutical preparations both for internal administration and for extermal application. The roots possess digestive and appetising properties when administered orally. Morever it shows curative effect in swelling and skin applications. From the literature on Chitraka it is clear that Chitraka acts as a digestant and appetiser due to plumbagin. Hence in the present investigation trials were run for the possible presence of selected enzymes in order to confirm the above qualities of Chitraka. This part of the study was to establish the presence of enzyme concentrations in the Chitraka root powder by both qualitative as well as quantitative methods.

(a) Both qualitative and quantitative methods were used for the enzyme assays. The root powder of plant (100 g) was suspended in equal amount of sterile saline or sterile phosphate buffer (pH 6.8–7) and mixed in a Warrying blender at

†Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431 004, India.

‡Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, India.

low temperature (0–4°C) for 10–12 min. The mixture was centrifuged, the supernatant collected and used for the enzyme assays.

(b) 10 g of dry root powder, was suspended in 10 mL of sterile saline or sterile 100 mL of phosphate buffer (pH 6.8–7.0) and sonicated for 10 min. in a Raython sonicator at 0–5°C and centrifuged. The clear supernatant was used for different enzyme assays.

However, it is pertinent to stress here that both the above techniques give reliable and reproducible results. Both the supernatant assays used for the enzyme analysis were kept in a frozen condition in a sterile plastic bag. The enzymes were assayed according to the standard recommended methods. For reporting and recording the enzymes (Table-1), specific activity was calculated in terms of respective limits of enzyme(s) per milligram of dry weight of the roots used. The 23 enzymes assayed are listed in Table-1 with their names and their salient mechanism of action respectively. The enzymes selected for the present study were largely of two groups, *viz.*, hydrolases and oxido-reductases (Table-1) and also lysozyme (muramidase) lytic enzyme which falls largely under the hydrolytic enzymes.

TABLE-1
DIFFERENT ENZYMES ESTIMATED IN PLUMBAGINACEAE FAMILY

S. No.	Enzyme name	Mechanism of action(s)
(I)	*Hydrolases*	
1.	Amylase	Internal random hyrolase
2.	Amylase	Successive maltose units removed
3.	Glucoamylase	Hydrolysis of 1,4-glucan links
4.	Cellulase	Hydrolyzes β–1-4-glucan links in cellulose.
5.	Isoamylase	Hydrolysis of D-glucoside branch linkages in glycogen amylopectin and β-limit dextrin
6.	Hexokinase	ATP + D-hexose – ADP + D-hexose 6 PO_4
7.	Glucokinase	ATP – D-Glucose – ADP – D-Gl-6 PO_4
8.	β-D-Glucosidase	Hydrolysis of terminal non-reducing 1.4-linked D-glucose residues with relase of β-D-glucose.
9.	α-β-Galactosidase	Hydrolysis of terminal non-reducing α-D-galactose residues in α-D-galactosides including galactoses, oligosaccharides.
10.	Lactase (β-D-galoctosidase)	Lactose – H_2O – 2-D-glucose + β-D-galactose Hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides.
11.	Invertase (sucrose)	Sucrose + H_2O → D-glucose + β-D-fructose.
12.	Lipase	Triglyceride + H_2O → glycerol + fatty acids.
13.	Carboxyl esterase (esterase)	A carboxylic ester + H_2O → an alcohol + a carboxylic acid anion.
14.	Glucose-5-PO_4	D-Glucose-6-PO_4 + H_2O →D-Glucose + orthophosphate.

S. No.	Enzyme name	Mechanism of action(s)
15.	Phospholipase	A phosphotidyl choline + $H_2O \rightarrow$ 1,2-diacylglycero + choline phosphate.
16.	Proteases	
	(a) Caseinase	Casein + $H_2O \rightarrow$ Para-casei
	(b) Gelatinase	Gelatin + $H_2O \rightarrow$ Peptone
17.	Glucose-6-isomerase	
18.	Collagenase	Hydrolysis of peptide cleaves preferentially and bound in native collagen leaving an N-terminal (75%) and C-terminal (25%) fragment.
19.	Lysozyme	Hydrolysis of 1,4-β linkages between N-acetylinuramic acid and 2-actamide 2-deoxy-D-glucose residues in a mucopolysaccharide or mucopeptide.
(II) Oxido-reductases		
20.	Catalase	$H_2OP_2 + H_2O_2 \rightarrow O_2H_2O$
21.	Glucose oxidase	β-D-Glucose + $O_2 \rightarrow$ D-glucans + H_2O_2
22.	Peroxidase	Donor + $H_2O_2 \rightarrow$ oxidized donor $2H_2O$
23.	Glucose dehydrogenase	β-D-Glucose NAD($P^{+''}$) D-glucose-8-lactose + NAD(P)H.

It is quite evident from Table-1 that all four species of *Plumbago* Linn estimated enzymes have different mechanism of actions. However, it seems that *Plumbago zeylanica* and *Plumbago rosea* are more powerful in general or as for as the enzyme content *in vitro* is concerned, compared to other species, *viz.*, *Plumbago capensis* and *Plumbago albus* respectively.

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