

INTRODUCTION:

Picrorhiza kurroa Royle ex Benth (Family: Scrophulariaceae) is a perennial herb, growing primarily in the north-west Himalayan mountains. Rhizomes and roots of this plant are widely used for the treatment of a range of liver diseases (1 & 2). It is reported to have anti-cancer activity (3) and extracts can be used as selective enhancers of neuron growth (4,5)

The active constituents responsible for the medicinal properties of *P. kurroa* are mainly picroside-I and picroside -II (Figure 1) but little is known about the biosynthesis of these iridoid compounds. The comprehensive analysis of the synthesis of these compounds has been hindered by the lack of rapid, sensitive identification and quantitation methods for picrosides that are also suitable for the screening of large number of plant samples. Here we report a rapid and sensitive method of picrosides identification and quantitation. We also present evidence that tissue picroside contents vary according to the stage of plant development in *P. kurroa* leaves and roots.

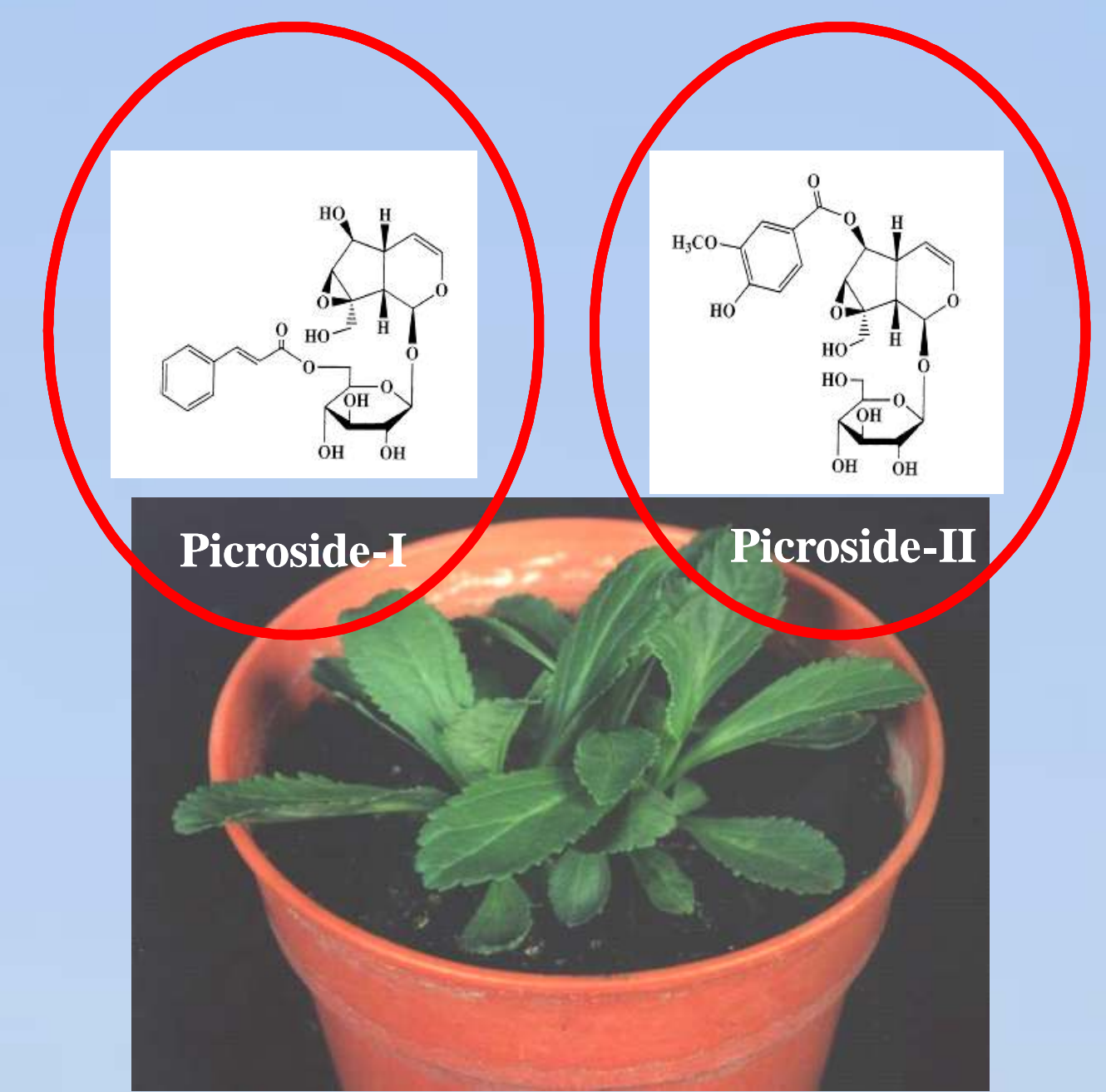


Figure 1: Structure of picrosides synthesised in *Picrorhiza kurroa* plants

Detection and quantitation of iridoids in *Picrorhiza kurroa*

Reverse-phase high performance liquid chromatography (HPLC) was used to identify iridoid compounds in plant tissues. *P. kurroa* leaf and root samples (75-100 mg) were ground to fine powder in liquid nitrogen. Iridoids were extracted in 80 % methanol (1.0 ml). Samples were centrifuged at 15000 g for 15 min and the supernatants filtered through 0.22 mm filters. The samples were then transferred to glass vials and HPLC analyses were performed.

HPLC was performed using a Waters 600 Controller module, Waters 996 photodiode Array detector and a Waters 717 plus Autosampler. A Phenomenex AQUA 5 micron 250X4.60 mm (synergy) column coupled to a 1 cm X 4.3 mm ODS guard column was used. All operations were conducted at room temperature.

A two solvent system, consisting of a mixture of 0.05 % trifluoroacetic acid (Solvent A) and a methanol/acetonitrile (1:1) mixture (solvent B) was used at a ratio of 70:30 (V/V). The column was eluted in the isocratic mode with a flow rate of 1.0 ml/min. Iridoid compounds were detected by their absorbance at 270 nm.

The identity of the picroside peaks was established by: 1, a comparison of retention times (Figure 2), 2. UV spectra (220 -350 nm; Figure 3) and 3. co-injection with standards.

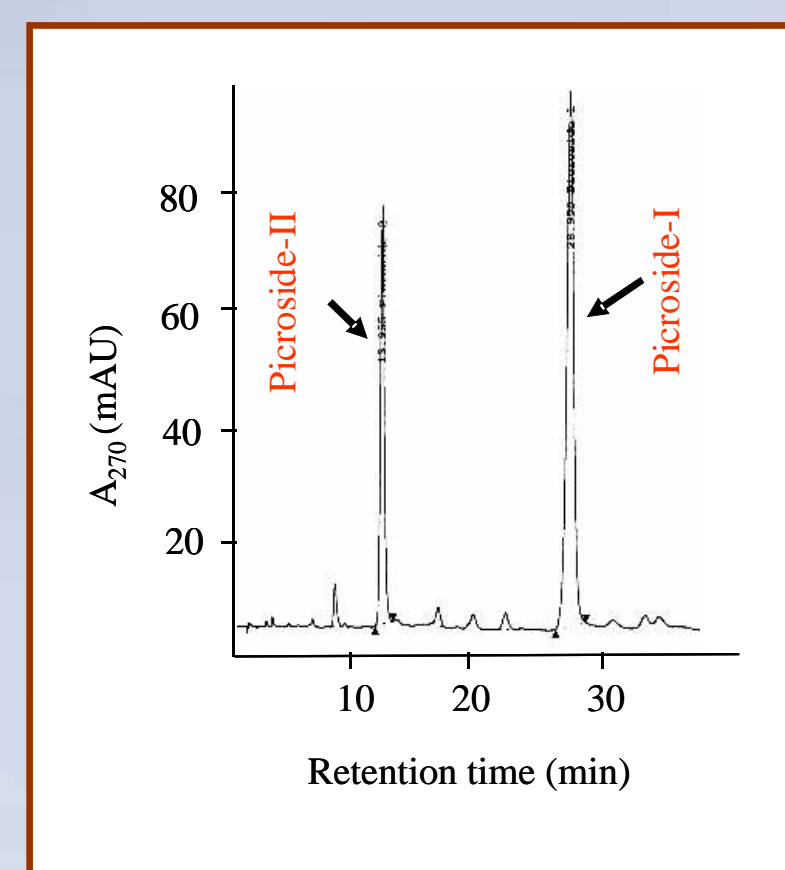


Figure 2: Elution profile of standard picrosides

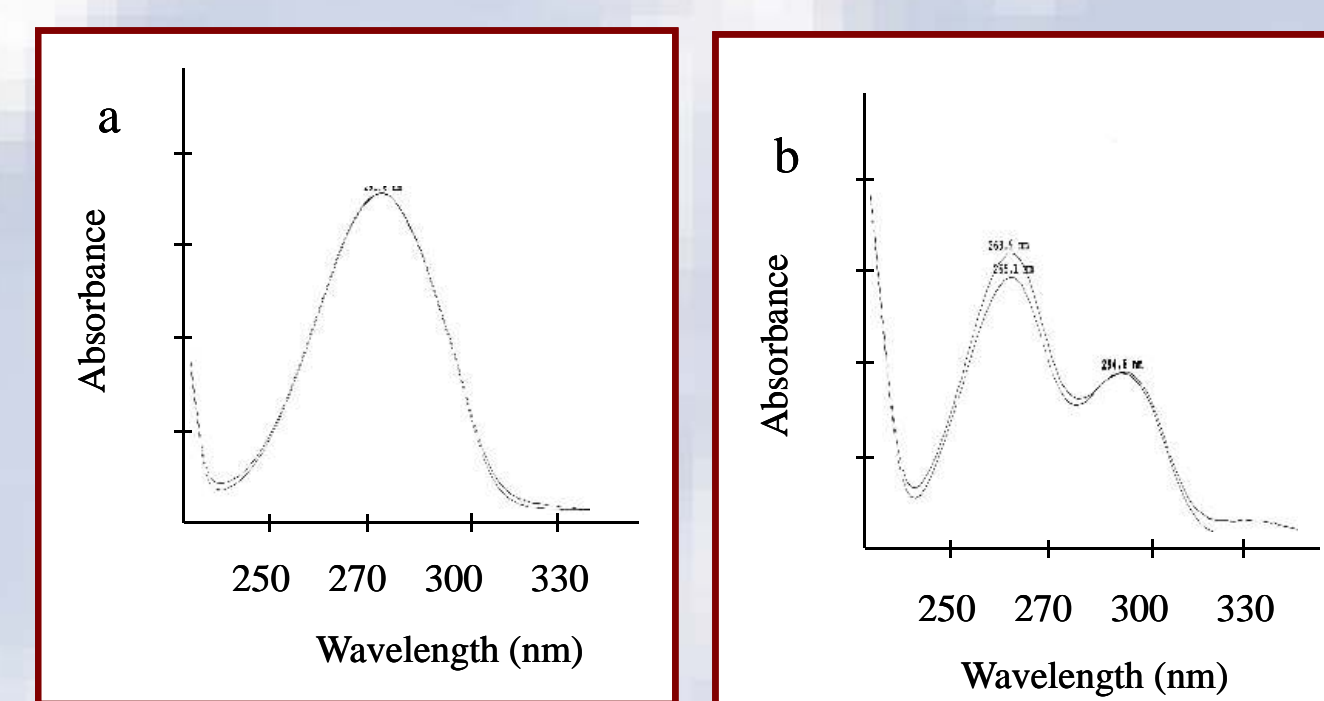


Figure 3: A comparison of the UV-spectra (220 to 350 nm) of the different picrosides(a- picroside I; b - picroside-II) extracted from *P. kurroa* with those of standards.

Leaf samples contained mainly picroside-I whereas root samples contained mainly picroside-II (Figure 4).

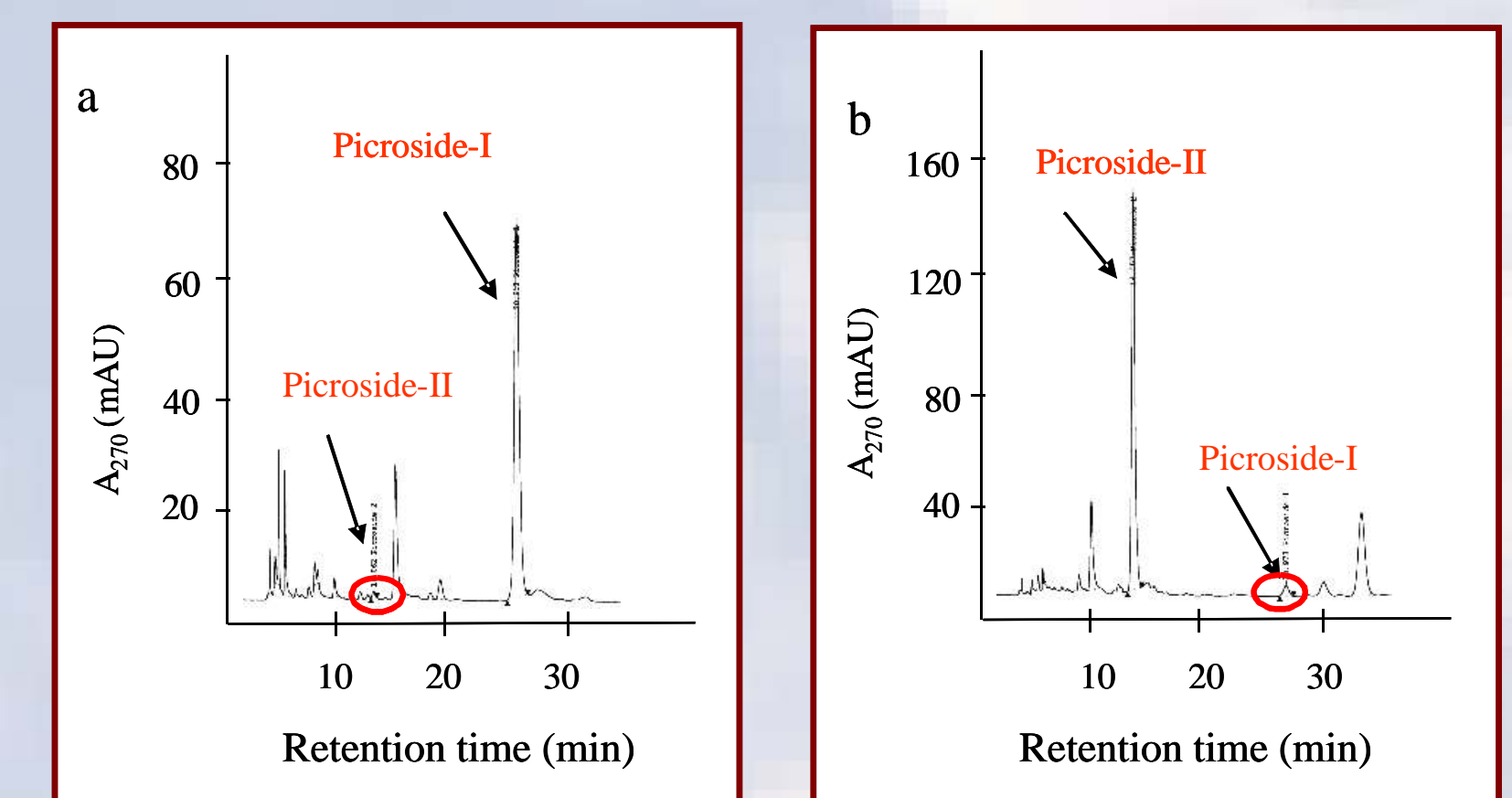


Figure 4: Iridoid compounds in *P. kurroa* tissues. leaf (a) and root (b) extracts

Picroside contents of *P. kurroa* plants at different growth stages

The amounts of picrosides present in tissue samples were quantified using the calibration curves prepared with standard picrosides. Four different stages of plant growth were examined.

During all four stages of plant growth picroside-I was the major iridoid compound present in leaves whereas picroside-II was predominant in roots (Table-1 and Figure 5).

Tissue	Picrosides (mg/g fw)							
	30 days		75 days		150 days		240 days	
	P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II
Leaves	16.2 (±0.75)	1.7 (±0.10)	9.1 (±0.01)	1.7 (±0.20)	15.7 (±0.61)	1.8 (±0.06)	3.1 (±0.06)	3.7 (±0.08)
Roots	2.9 (±0.09)	10.8 (±0.30)	1.1 (±0.05)	27.3 (±0.69)	0.8 (±0.01)	30.3 (±0.11)	1.7 (±0.02)	10.1 (±0.25)

Table1: Picroside contents of *P. kurroa* plants at different stages of growth

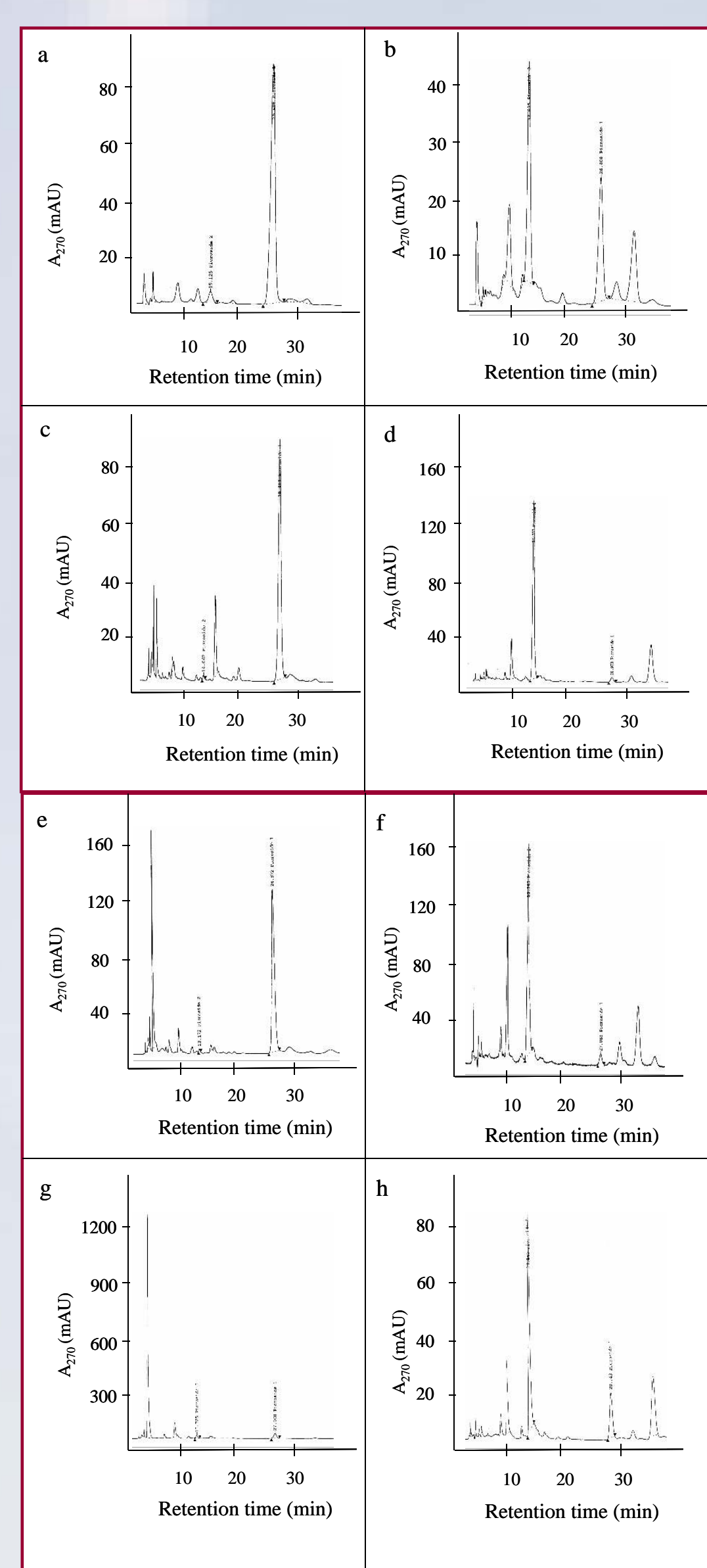


Figure 5: Elution profiles of *P. kurroa* extracts at different stages of growth. Leaf extracts at 30 (a) 75 (c) 150 (e) and 240 (g) days after planting. Root extracts at 30 (b) , 75 (d) , 150 (f) and 240 (h) days after planting.

Antioxidant status of *P. kurroa* tissues through development

Ascorbic acid and glutathione are the two main antioxidant buffers of plant cells. They play a vital role in protecting plants against oxidative stress.

There is comparatively more ascorbic acid in *P. kurroa* leaves than roots, while roots contain more glutathione than leaves (Figure 6).

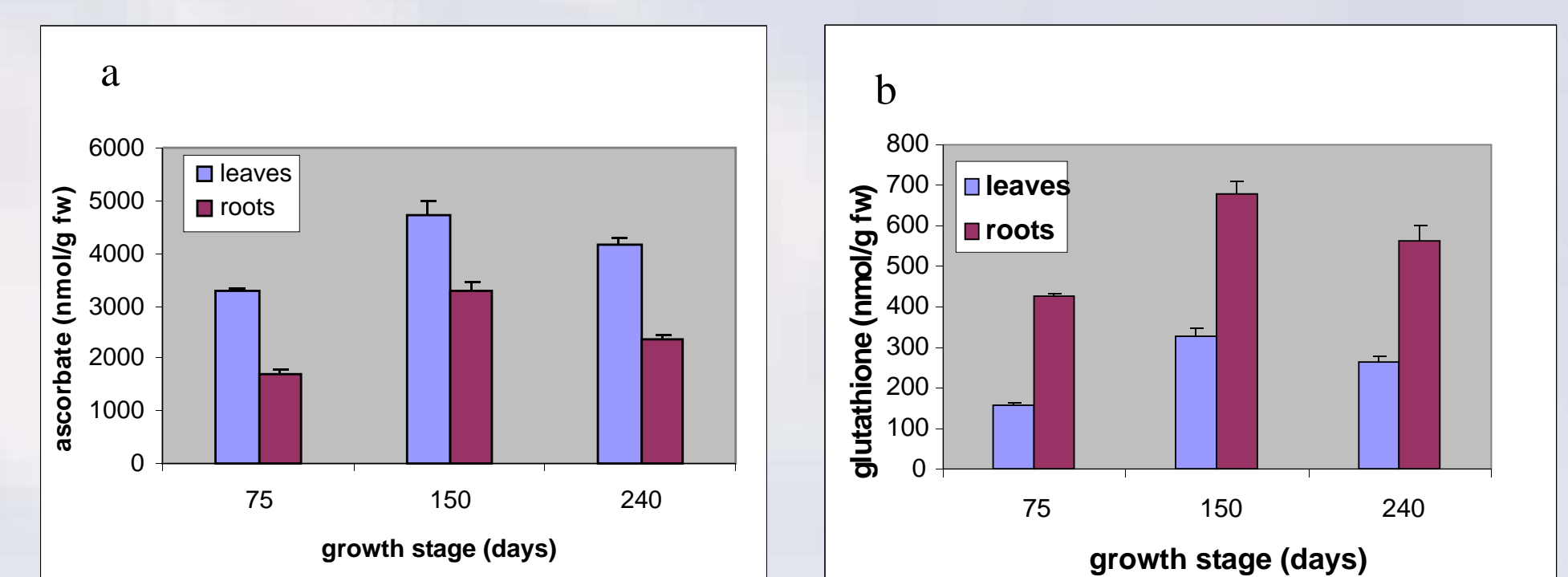


Figure 6: The ascorbate (a) and glutathione (b) contents of *P. kurroa* tissues during development.

Conclusions and perspectives:

- Leaves and roots show differential accumulation of picrosides. Picroside-I accumulated in leaves whereas roots contained higher amounts of picroside-II.
- Leaves and roots show differential accumulation of antioxidants. Ascorbate was more abundant in leaves than roots whereas glutathione is most abundant in roots.
- This analysis of picroside contents in leaves and roots provides the first indication of differential metabolism of Picrosides in these plant organs. This provides the foundation for comparative analysis of biosynthesis and transport between tissues.

Reference:

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