

PHYTOLACCA ACINOSA ROXB., AN ADULTERANT OF INDIAN BELLADONNA

BY K. L. KHANNA AND C. K. ATAL

From the Department of Pharmacy, Panjab University, Chandigarh, India

Received January 19, 1960

Pharmacognostical data of the leaves and roots of *Phytolacca acinosa* Roxb. has been collected and compared with that of *Atropa acuminata* Royle ex Lindley.

ROOTS and leaves of *Phytolacca americana* have long been used as an adulterant¹ of belladonna in European and American markets. Roots and leaves of *P. acinosa* have been reported² to have formed an adulterant in certain consignments of Indian belladonna shipped abroad. Over several years, we have repeatedly come across samples of Indian belladonna leaves and roots adulterated with the leaves and roots of *P. acinosa*. A detailed study of the adulterant was therefore undertaken to distinguish it from true Indian belladonna.

Phytolacca acinosa Roxb. (Family Phytolaccaceae) is a glabrous shrub with succulent hollow stem. It bears oblong, dark green alternate leaves. The flowers are pale green, arranged on a racemose inflorescence. The plants possess a thick fleshy branched tap root. It is a plant originating from China and it is found wild or cultivated in the temperate Himalayas from Hazara and Kashmir to Bhutan at an altitude of 5,000 to 9,000 ft. It is called "matazor" in Hindi and "sarangun" in Urdu. It contains³ a bitter toxic substance, phytolacca toxin. No detailed chemical investigation of the plant has been reported. In the indigenous system of medicine, the plant is described as narcotic and the oil from the root is used to allay pain in joints. The leaves are cooked and eaten by the village folk as a vegetable. It is supposed that the toxic principles are destroyed during cooking.

MATERIAL AND METHODS

Between 1953 and 1959, three samples of roots and leaves were obtained from different drug dealers under the name of Indian belladonna. These samples were found not to belong to either *Atropa acuminata* or *Atropa belladonna*. In 1959, an authentic specimen of *Phytolacca acinosa* Roxb. was collected from Kashmir and was found to be identical with the adulterant. For macroscopical descriptions the commercial drug was used whereas for histological work commercial as well as fresh authentic specimens preserved in 50 per cent formalin : acetic acid : ethanol (5 : 5 : 90) were used. The usual methods of paraffin embedding and sectioning on a rotary microtome were followed. Staining was with saffranin and fast green.

THE ROOT

Macroscopy

The individual pieces (Fig. 1) in a drug sample vary greatly in their appearance. Those from the root crown (Fig. 1A) are irregular, knotty

and show occasionally the remains of aerial shoots. Such pieces are up to 10 cm. long and 3 to 5 cm. thick. The roots are frequently longitudinally cut and are twisted or curved. They are 1.5 to 3 cm. wide and 5 to 11 cm. long. The transversely cut and smoothed end of such roots (Fig. 1B) shows characteristic concentric rings of xylem which become more prominent on treatment with phloroglucinol and hydrochloric acid reagent. The longitudinally cut surface (Fig. 1C) exhibits vertical

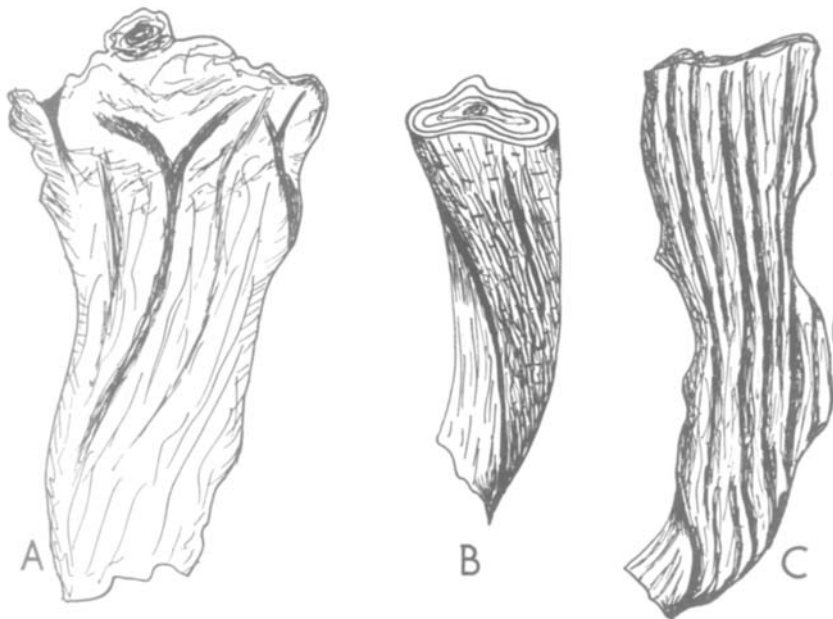


FIG. 1. Commercial roots $\times \frac{1}{2}$. A, root crown; B, root showing longitudinal wrinkles, lenticels, twisted appearance and concentric rings of xylem at the transversely cut end; C, longitudinally cut view of root.

lighter coloured ridges. The external surface is light brown in colour and shows longitudinal wrinkles and transversely elongated lenticels. The roots have a short fracture and an indistinct taste and odour. A comparison with *A. acuminata* is made in Table I.

Microscopy

The root in transverse section (Figs. 2-3) shows from without inward, the cork, the secondary cortex and an abnormal stele composed of open collateral vascular bundles arranged in concentric rings.

The cork (Fig. 4) consists of 5 to 7 layers of rectangular, suberised and slightly lignified cells. The radial walls of these cells are wavy and measure T, 17-42-70 μ ; R, 11-14-28 μ . They appear rectangular to polygonal in surface view. The phellogen is a single layer of thin-walled cells measuring T, 38-63-105 μ ; R, 6-9-25 μ .

The secondary cortex is composed of about 15-16 layers of parenchyma cells which show well marked intercellular spaces. The first 5 to 6 layers

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of cells are much more elongated tangentially and are rectangular in outline. The cells measure T, 35–71–157 μ ; R, 29–39–53 μ . The next 10 or 11 layers of cells are smaller in size measuring T, 38–61–109 μ ;

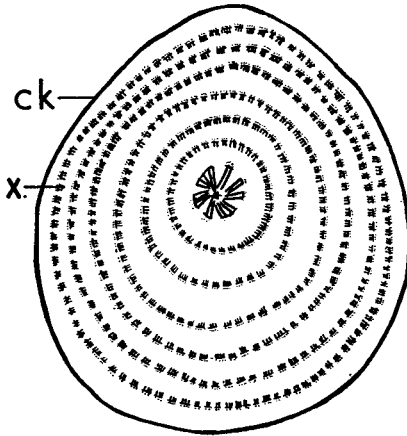


FIG. 2. T.S. root (diagrammatic) original size. ck, cork; x., xylem.

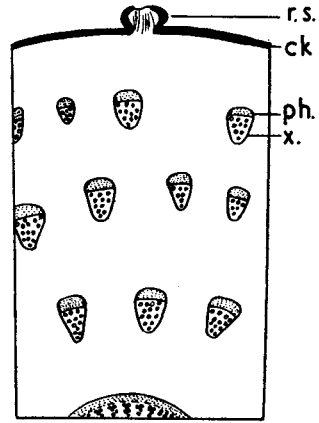


FIG. 3. A sector of the root in t.s. (diagrammatic) $\times 5$. ck, cork; ph., phloem; x., xylem; r.s., root scar.

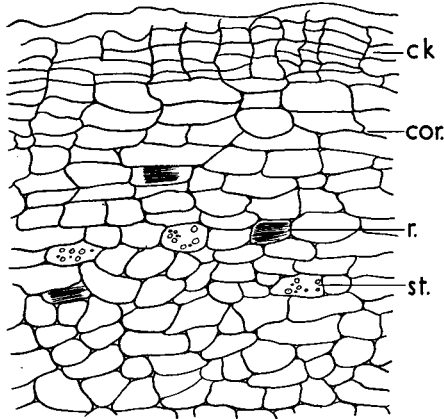


FIG. 4. T.S. root showing cork and secondary cortex $\times 60$. cor., cortex; ck, cork; r., raphides; st., starch grains.

R, 32–49–73 μ and are arranged irregularly. Some of these cells contain starch grains and raphides of calcium oxalate.

The vascular bundles (Fig. 5) arranged in concentric rings are open and collateral. The phloem consists of sieve tubes, companion cells and phloem parenchyma. The cells of the phloem parenchyma are thin-walled and are devoid of intercellular spaces. Beneath the phloem there is a single layer of thin-walled cambium cells.

TABLE I

DISTINGUISHING FEATURES OF THE ROOTS OF *Phytolacca acinosa* AND *Atropa acuminata*

Character	<i>P. acinosa</i>	<i>A. acuminata</i>
Form	Variable—mostly longitudinally cut and twisted	Variable—mostly transversely cut and cylindrical
Outer surface	Prominent lenticels and longitudinal wrinkles	Lenticels few and wrinkles less prominent
Transverse surface	Concentric rings of xylem	Radiate xylem
Longitudinally cut surface	Marked light coloured longitudinal ridges	No longitudinal ridges
Taste	Indistinct	Bitter

The stele is traversed by multiseriate vascular rays, the cells are thin-walled, parenchymatous, radially elongated and contain abundant starch grains. The cells measure T, 27–47–66 μ ; R, 45–60–105 μ . The xylem is formed of xylem vessels and xylem parenchyma. It is devoid of tracheids and fibres. The xylem vessel segments show reticulate thickening and have porous end walls. In macerated preparations, they measure 65–150–240 μ in length and 37–93–111 μ in breadth.

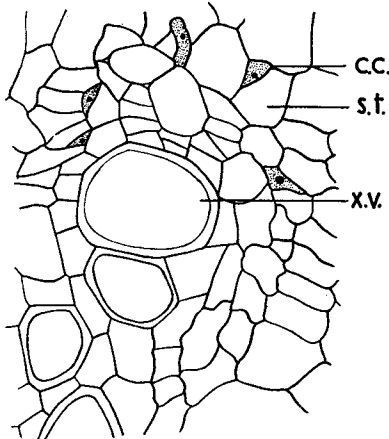


FIG. 5. T.S. portion vascular bundle $\times 148$. c.c., companion cell; s.t., sieve tube; x.v., xylem vessel.

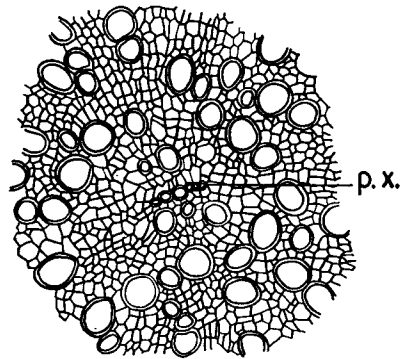


FIG. 6. T.S. root showing primary xylem $\times 72$. p.x., primary xylem.

The primary root (Fig. 6) is diarch and shows normal growth, thus forming secondary xylem and secondary phloem.

Starch consists of simple and 2 to 5 compound grains. They are mostly circular but sometimes distorted in outline and show a dotted or cleft hilum in the centre. They measure up to 45 μ in diameter. Calcium oxalate crystals are found in all the parenchymatous tissues in the form of raphides which measure T, 35–42–87 μ ; R, 7–11–18 μ .

THE LEAF

Macroscopy

The leaves are oblong (Fig. 7A) as compared to belladonna leaves (Fig. 7B) which are ovate in outline. The medium-sized leaves measure

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about 15 cm. in length and 5 cm. in breadth. However, in some samples, leaves up to 30 cm. in length may be seen. The margin is slightly wavy and toothed towards the upper half of the lamina. The apex is acute with a distinct apiculus and the base is asymmetrical. The lateral veins arise from the midrib at an angle of about 60° and anastomose near the margin. The commercial samples of leaves occur in a crumpled state

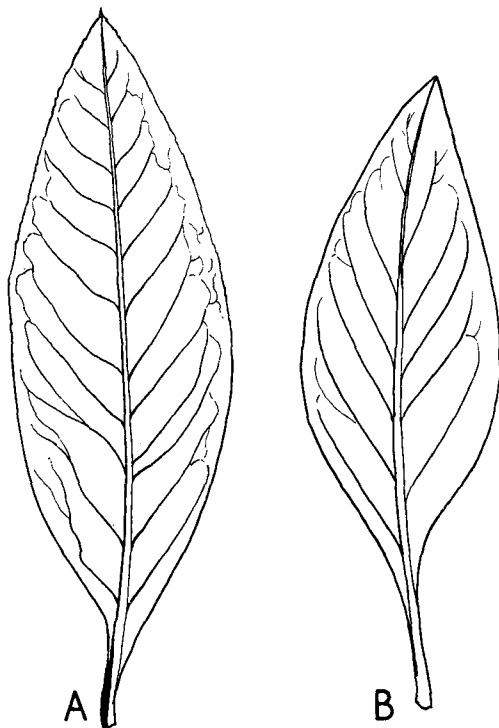


FIG. 7. Leaf $\times \frac{1}{2}$. A, *Phytolacca acinosa*;
B, *Atropa acuminata*.

and have a thin membranous texture. If flattened leaves are held against the light numerous tiny scattered specks representing raphides may be visible to the naked eye. Odour and taste are indistinct.

Microscopy

The midrib (Fig. 8) in transverse section shows an arc-shaped meristele. The meristele is composed of 5 to 6 groups of open collateral vascular bundles which do not appear separate when the section is cut near the apex. The endodermis and pericycle are not very clear. The rest of the midrib is filled with thin-walled polygonal parenchyma cells except the upper and lower hypodermal regions which have a layer of collenchyma 2 to 3 cells thick. The cells of the parenchyma contain raphides of calcium oxalate.

The upper epidermal cells are rectangular to squarish in outline in a transverse section. The cells show straight anticlinal walls, but the outer tangential walls of some cells show papillose outgrowth which is much more prominent in the region of the midrib and lateral veins. These papillae contain some granular matter which is insoluble in chloral hydrate

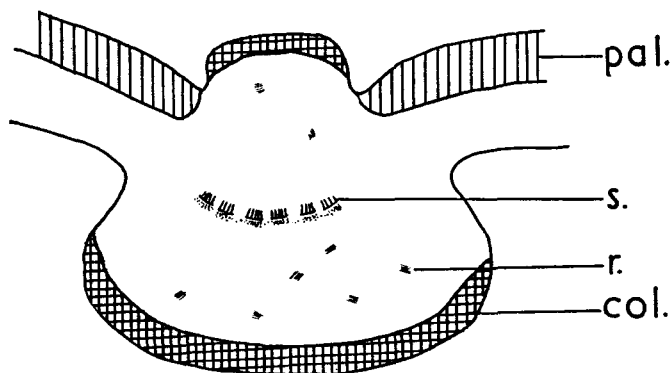


FIG. 8. T.S. leaf (diagrammatic) $\times 45$. col., collenchyma; pal., palisade cells; s., stele; r., raphides.

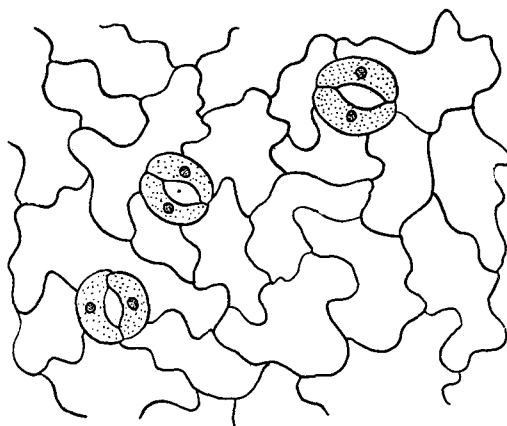


FIG. 9. Surface view of lower epidermis $\times 380$.

and concentrated hydrochloric acid. The upper epidermis has an indistinct cuticle and rarely shows stomata, which if present are of the anomocytic type. In surface view the upper epidermal cells are polygonal with straight walls. The palisade layer is single cell thick and is discontinued at the midrib. The spongy parenchyma is 5 to 7 cells thick and shows intercellular air spaces. The lower epidermis also has an indistinct cuticle. The cells show papillae similar to those found on the upper epidermis, and there are numerous anomocytic stomata present. The cells show wavy anticlinal walls in surface view (Fig. 9).

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The values of quantitative microscopic determinations taken from ten readings are as follows.

Vein islet Number	1-1.25-2.5
Stomatal Index	14.7-14.9-15.2
Stomatal Number	28-48-54
Palisade Ratio	1.7-2-3

The vein islet pattern of *P. acinosa* and *A. acuminata* was also found to be a distinguishing feature. These quantitative values have been compared (Table II) with those of *A. acuminata* reported by Wallis⁴.

TABLE II
COMPARATIVE STUDY OF THE LEAVES OF *P. acinosa* AND *A. acuminata*

Character	<i>P. acinosa</i>	<i>A. acuminata</i>
<i>Macroscopy</i>		
Size	15-30 cm. long 5-7 cm. broad	6-20 cm. long 3-11 cm. broad
Outline	Oblong	Ovate
Apex	Acute, distinct apiculus	Acute, no apiculus
Margin	Slightly toothed	Entire
Texture	Papery	Tough
Angle of lateral veins	60°	45°
Trichomes	Absent	Present
<i>Microscopy</i>		
Stele of the midrib	Collateral	Bicollateral
Crystals	Raphides	Microsphenoidal
Cuticle	Indistinct	Distinct
<i>Quantitative values</i>		
Vein islet number	1-1.25-2.5	3-4.2-5.1*
Stomatal index	14.7-14.9-15.2	16.7-17.6-18.8
Stomatal number	28-48-54	62.5-93-174
Palisade ratio	1.7-2-3	4.0-8.0-13.5

* The vein islet number of *A. acuminata* has not been reported previously and was determined by the authors.

CONCLUSION

On the basis of the description of *P. acinosa* outline it is clear that this adulterant can be easily distinguished in commercial samples. The distinguishing features are presented in Tables I and II.

Acknowledgement. We thank Dr. K. N. Gaind, Head of the Department of Pharmacy, Panjab University for facilities and encouragement.

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