Pharmacognostical studies on the root of *Apama siliquosa* Lamk. (Aristolochiaceae)

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Abstract

The roots of *Apama siliquosa* Lamk. have many medicinal virtues. The correct nomenclature and botanical description of the taxon along with pharmacognostical details of the root and the diagnostic features of the drug are described with suitable illustrations.

1. Introduction

The genus *Apama* Lamk. include 12 species of which 3 are native to the subcontinent of India.\(^1\) *Apama barberi* Gamb., is endemic to Tirunelveli hills (Tamil Nadu) in South India;\(^2\) *A. siliquosa* Lamk., occur throughout the evergreen forests of W. Ghats\(^3\) while *A. dalzelii* (Hk. f.) 0. Ktze., (*=Bragantia dalzelii* Hk. f.) doubtfully occurs in Concan\(^4\) and hence finds a place under 'excluded species'. There has been no subsequent report of *A. dalzelii*

The mature roots of *A. siliquosa* are reportedly used by Ayurvedic physicians of Konkan and Malnad districts of Karnataka for the treatment of dysentery; the roots are ground to a paste with lemon juice (60 gm in 14 ml) and administered orally every 15 minutes; the roots are said to be efficaceous in cholera; it is also claimed that an ointment prepared from the plant is beneficial in the treatment of carbuncles and inveterate ulcers.\(^5\) Like other plants belonging to the family Aristolochiaceae, it is supposed to have virtues in the cure of snakebites and is regarded as one of the most powerful antidotes to poison known on the west coast.\(^6\) The roots contain an alkaloid Chakranine.\(^7\)

Some pharmacognostical studies on the root of this taxon under the name *Bragantia wallichii* R. Br. has been carried out by Pratap Singh\(^8\) and Deshpande and Pandit.\(^9\) Since this work was found to be of a preliminary
nature, a detailed investigation was undertaken, the results of which are presented in this paper with suitable illustrations. The scope of the present study include the following: (1) the correct nomenclatural status of the genus and species; (2) brief botanical description of the taxon and citation of voucher herbarium specimens examined; (3) macro and microscopic characters of the root; (4) phytochemical studies comprising investigations on physical constants, fluorescence analysis, organic analysis including chromatography and (5) diagnostic features for identification of the drug.

2. MATERIALS AND METHODS

Fresh samples of the roots of *A. siliquosa* were collected from Cherambady forests, Gudalur Taluk, Tamil Nadu, during Medico-botanical survey tour to Nilgiris District in the month of October–November, 1975. These were fixed in 70% alcohol according to Wallis. Microchemical studies were performed according to Kay and Johansen. For chemical and fluorescence studies, the shade dried roots were powdered and filtered through a sieve of 60 mesh and analysed according to Peach and Tracey and Chase and Pratt. Physical constants and organic analysis have been carried out following methods mentioned in the *Indian Pharmacopoeia*.

3. NOMENCLATURE AND BOTANICAL DESCRIPTION

The genera *Apama* Lamk. and *Bragantia* Lour. are congeneric. The genus *Apama* (1783) is validly published earlier and hence has priority over the genus *Bragantia* (1790). Accordingly, the correct name of the genus as per rule of priority of the *International Code of Botanical Nomenclature* (1972) should be *Apama* and not *Bragantia*. The first validly published specific name for the taxon under *Apama* is *A. siliquosa* and hence should be taken as the correct name for the taxon. The correct nomenclature and citation of the taxon thus should be as: *Apama siliquosa* Lamk. *Encycl. 1*: 91. 1783; Schmidt in *Pfam.* (ed. 2) *16 b*: 234. 1935; Gamb. Fl. Pres. Madras 840 (repr. ed. 1967). *Bragantia wallichii* R. Br. ex Wt. and Arn. *Edinburgh. New Philos. J.* 181. (1933); Hk. in Hk. f. et al., *Fl. Brit. India 5*: 73. 1886; Cooke, *Fl. Pres. Bombay 2*: 15 (repr. ed. 1967); *Wt. Icon. t.* 520. 1842: *Niruvate, Chakrapani beru* (Kannada).

Erect shrubs, 1·5–3 m tall; stems reddish to purple; branches many, angled, smooth, swollen at internodes, glabrous or nearly so; internodes long. Leaves 6–18 × 2·5–7·5 cm, alternate, subsessile, oblong-lanceate, glabrous above, minutely pubescent beneath, long acuminate at apex, rounded or acute at base. Flowers 8 mm across, pink, in axillary, irregular cymes;
pedicels 1–1·2 cm long, slender, densely hairy. Capsules up to 8 cm long linear, 4-gonous, minutely pubescent; seeds 3-gonous, pitted (Plate I, figures A–C).

Herbarium specimens examined: Yoganarasimhan 1013, 20-1–1972, hair pin bend No. 9, Charmadi Ghats, Chikmagalur District, common in evergreen forests, c 730 m, flowering and fruiting; Yoganarasimhan 1729, 22-11–1973, Tungabhadra state forests, Kerekatte, Chikmagalur District, fairly common in evergreen forests, c 1000 m, flowering and fruiting; Yoganarasimhan 1977, 28-11–1974, on the way to Gangamula, Kerekatte, Chikmagalur District, fairly common in evergreen forests, c 1000 m, flowering and fruiting; Yoganarasimhan 2216, 12-11–1975, Nadugani to Devala, Gudalur Taluk, Nilgiris District, fairly common in evergreen and shola forests, c 1300 m, flowering and fruiting; all the specimens are deposited at the Herbarium, Regional Research Centre, Bangalore.

4. Macroscopic Characters

The roots are 1 to 1·5 m or more long, somewhat curved and tapering towards the ends, varying in thickness from 15–30 mm across. Their external surfaces are smooth with deep longitudinal fissures giving them the appearance of islands with tapering ends; a few rootlet scars are present. They are greyish-cream coloured externally and internally yellowish-white. The bark peels off easily in mature roots. In transverse section, broad yellowish medullary rays extend towards the centre. While there is no characteristic smell, the taste is bitter. Fracture is brittle and rough (Plate I, figure B).

5. Microscopic Characters

The roots are nearly circular in transverse section and regular in outline with a prominent central stelar portion interspersed by radiating medullary rays (Plate II, figure 1). The cork is 15 to 20-layered in thickness; the cells are cubical to rectangular, thin-walled and arranged in radial rows and measure \( T = 14–20–23·8 \times 10–14–20 \mu \) (Plate II, figures 2 and 6d), rupturing at intervals to form lenticels. The cork cambium is single-layered, thin-walled and rectangular (Plate II, figure 2). The secondary cortex is 6 to 9-layered and consists of thin-walled isodiametric to tangentially elongated cells (Plate II, figure 2). The stone cells are few, scattered, thick-walled, varying in size and shape, some are elongated and others are oval with a narrow lumen, measuring \( T = 47·6–54·4–68·4 \times 20–26 \mu \), \( M = 105–225–330 \times 15–30–37·5 \mu \) (Plate II, figures 2 and 6a, c). The secondary phloem is many-layered and consists of sieve tubes, companion cells and phloem parenchyma (Plate II, figure 3). A single row of rectangular
Figures A–C. A. Flowering twig; B. External and internal surface of the root; C. Fruit.
Figures 1-8: 1. T.s. of root (diagrammatic); 2. Portion of cork enlarged; 3. Portion of secondary phloem enlarged; 4. T.s. of wood; 5. L.s. of wood; 6. Bark macerate: a. and c. stone cells; b. ray cells; d. cork cells; 7. Wood macerate: A. tracheids; B. fibres; C. vessels; D. ray cells; 8. Powder drug: a. starch grains; b. cork cells; c. ray cells; d. bits of fibres; e. stone cells; f. vessels; g. tracheids.

(Abbreviations: C, cork cells; CA, cambium; CC, Cork cambium; F, Fibres; MR, Medullary ray; PX, Protoxylem; SC, Stone cells; SEC, Secondary cortex; SP, Secondary phloem; T, Tracheids; V, Vessels.)
thin-walled cambium is present-between phloem and xylem (Plate II, figure 3). The xylem is six-armed, radiating with broad medullary rays interspersing. Xylem consists of vessels, tracheids, wood fibres and wood parenchyma. The vessels vary in size and shape and possess transverse oblique perforations and simple pits on their walls; some are short and wide while others are narrow and elongated and measure \( T = 26.6-39.9-53.2 \mu m \), \( M = 150-225-270 \times 30-45-75 \mu m \) (Plate II, figures 4, 5 and 7). Most of the vessels have tail-like ends beyond their oblique end walls. Tracheids are narrower with tapering ends; some are elongated with bordered pits; they measure \( M = 120-150-270 \times 15-23-30 \mu m \). The wood fibres are many, longer than the tracheids, thick-walled with pointed ends and much reduced pits, measuring \( M = 675-825-1425 \times 15-16-20 \mu m \) (Plate II, figures 7 A, B, C). Medullary rays are multiseriate, 6-12-layered, broadly wedge-shaped with wider ends towards periphery (Plate II, figure 4); the ray cells are thin-walled, rectangular to oval, measuring \( T = 19.9-26.6-39.9 \times 19.9-26.6 \mu m \) (Plate II, figures 6 b, 7 D) and are filled with simple, oval to rounded starch grains which measure \( T = 6-8-12 \mu m \) in diameter. Pith is absent and the central portion is occupied by protoxylem (Plate II, figure 1).
CELL CONTENTS:—The cork cells contain dark brown contents of tanniferous nature. The cork cells contain oil globules also which give pink colour on treatment with Sudan III. Starch grains are plenty in medullary rays and few in secondary xylem and cortical regions.

POWDER ANALYSIS:—The powder is yellowish-green with bitter taste. The powder filtered through a 60 mesh sieve and mounted in chloral hydrate shows the presence of starch grains, cork cells, ray cells, fibres, stone cells vessels and tracheids (Plate II, figures 8a-g).

6. PHYTOCHEMICAL STUDIES

The phytochemical studies have been carried out as per methods mentioned earlier.

A. Physical constants

1. % loss on drying at 110°C .. 10.86
2. % total ash .. 8.70
3. % water insoluble ash .. 7.90
4. % acidity with 0.1 N NaOH .. 0.5 ml
5. % acid insoluble ash .. 4.70
6. % crude fibre .. 32.15
7. % extractive principles:
   (a) Hexane .. 0.80
   (b) Ether .. 0.84
   (c) Benzene .. 1.04
   (d) Chloroform .. 0.04
   (e) Ethanol .. 4.40
   (f) Water .. 5.60
8. % solubility:
   (a) Water .. 4.56
   (b) Ethanol .. 4.96
B. Fluorescence analysis:

<table>
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<th>Treatment</th>
<th>Results</th>
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<tbody>
<tr>
<td></td>
<td>Ordinary light</td>
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<tr>
<td>1. Powder as such</td>
<td>Greyish-yellow</td>
</tr>
<tr>
<td>2. Methanol</td>
<td>Dark green</td>
</tr>
<tr>
<td>3. Ethanol</td>
<td>Grey</td>
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<tr>
<td>4. Carbon tetrachloride</td>
<td>Dark grey</td>
</tr>
<tr>
<td>5. 0·1 N NaOH (Methanolic)</td>
<td>Brown</td>
</tr>
<tr>
<td>6. 0·1 N HCl</td>
<td>Light grey</td>
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C. Organic analysis.—Manjunath et al.,\textsuperscript{17} isolated from the roots of this plant a fatty oil consisting mainly of palmitic, lignoceric, oleic and linolic acid and a yellow bitter principle identical with iso-eristolochic acid. Kamat et al.,\textsuperscript{18} isolated from the roots pure crystalline alkaloidal principle Chakrenine C_{21}H_{34}O_4 NCL, m.p. 235\degree C.

In the present study 250 gms of \textit{A. siliquosa} roots were successively extracted with petroleum ether, chloroform, EtOH and water. Petroleum ether extract answered for steroids and carboxylic acids. Chloroform extract answered for steroids, triterpenoids and carboxylic acids. Ethanolic extract answered for carboxylic acids, tannins, sugars and alkaloids. The aqueous extract answered for carboxylic acids, tannins, sugars and alkaloids. The first two fractions were submitted to TLC using Benzene-chloroform-ethyl acetate (2: 2: 1) as eluent and FeCl_3 as developer (text-figure 1). The EtOH and water extract were hydrolysed with 4N H_2SO_4 and worked up for alkaloid. The alkaloidal fraction in acetone was submitted to ascending paper chromatography using BuOH : ACOH : H_2O_4 (4: 1: 5) as the solvent and Dragendorff’s reagent as spraying reagent. One bright orange spot was obtained with Rf value 0·8445

7. Diagnostic Characters of the Drug

The following characters can be considered as diagnostic features in the identification of the drug: (1) external surface of the root is smooth with deep longitudinal fissures giving the appearance of islands with
tapering ends; (2) presence of oil globules in the cork region; (3) presence of starch grains in abundance in the ray cells; (4) presence of a prominent central stelar portion interspersed by radiating medullary rays.

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REFERENCES