Natural Product Sciences
9(4) : 286-290 (2003)

Pharmacognostic Evaluation of Ratanjot-*Arnebia nobilis* Rech. f.

Sayyada Khatoon, B. N. Mehrotra and Shanta Mehrotra*

Pharmacognosy and Ethnopharmacology Division
National Botanical Research Institute, Lucknow, India

Abstract – Ratanjot is attributed to eight species of Boraginacae species belonging to genera *Alkanna*, *Arnebia*, *Maharanga* and *Onosma* and regarded as one of the important herbal drugs of indigenous systems of medicine. The root and root stock, which form the actual drug, are considered to be an anthelmintic, antipyretic and antiseptic. They are also claimed to be useful in burn, eczema, wounds and eruptions, and used for treating the diseases of eyes, bronchitis, abdominal pains, itch, etc. Several workers reported that the Naphthaquinones (arnebins), the main active constituents of the drug, are responsible for its colour and therapeutic efficacy. It is claimed that the *Arnebia nobilis* was imported to India from Afghanistan but the red coloured roots resembling with *A. nobilis* were found to be gathered by local people for commercial purposes during the course of botanical survey of Himalayan region. Hence, it is decided to evaluate *Arnebia nobilis* pharmacognostically. The important macro-microscopic features of this species are roots 1-5 cm broad; outermost xylem with broad vessels and innermost with groups of radially arranged narrow vessels while the middle region occupied by alternate rings of clusters of broad and narrow vessels and presence of pith. Besides the percentage of arnebin-1,-3 and 4 are 9.37, 10.53 and 1.72 respectively.
Keywords – *Arnebia nobilis,* Arnebin, Pharmacognosy, Ratanjot, Root, TLC.

Introduction

Ratanjot is popular not only in India but also in other Asian countries for imparting a pleasing red colour to food stuffs, oils, fats and medicinal preparations. It is used for burns, eczema and eruptions in traditional systems of medicine. It is also considered to be anthelmintic, anti-pyritic and anti-septic, and are useful for treating the diseases of eyes, bronchitis, abdominal pains, itch, etc. (Anonymous, 1950). Isohexenylnaphthazarins (naphthaquinones), the main active constituents of this plant, are responsible for its colour and therapeutic efficacy (Khatoon & Mehrotra 1994). They are reported to have the antimicrobial (Shukla *et al.,* 1969, Bhakuni *et al.,* 1969, Patel & Patel 1966), anticancer (Katti *et al.,* 1979), antitumor (Gupta & Mathur 1972) and wounds healing properties (Papageorgiou 1980).

The botanical identity of Ratanjot is confusing as 15 plant species are mentioned in the literature under this vernacular name. Eight species of *Alkanna, Arnebia, Maharanga* and *Onosma* of Boraginaceous are used as Ratanjot due to their red coloured root. Although Bole (1961, 1962) provided a convincing explanation that the genuine Ratanjot is *Arnebia nobilis* Rech. f., which was imported into India from Afghanistan but during the course of botanical survey and collection of plants from Himalayan region, we at Central Drug Research Institute, Lucknow observed that local collectors gathered the roots resembling Ratanjot in colour and texture from the areas for commercial sale (personal observation). Therefore, Khatoon *et al.* (1993) made an effort to authenticate the commercial samples obtained from drug markets and found that all the market samples were a mixture of two or three taxa, except for the Amritsar sample that was identified as *A. nobilis.* This is the common practice of any trade to find out cheap, similar looking substances for adulteration or as substitute to the genuine item for easy monetary gain. Hence, it is necessary to provide some quality control markers of *A. nobilis.* Keeping this in view, the detailed pharmacognostic evaluation of *Arnebia nobilis* was carried out. Study includes the macroscopic morphology, microscopic anatomical features, physico chemical parameters and TLC fingerprint markers of the root of *A. nobilis.*

Materials and Methods

Roots of *A. nobilis* were collected from the barren hilly slopes of Istalif, Afghanistan and voucher specimen number AF 43 was deposited in the Herbarium of Botany Division, Central Drug Research Institute, Lucknow, India. The collected

*Author for correspondence
Fax: 91-522-2207219, E-mail: pharmacognosyl@radiffmail.com

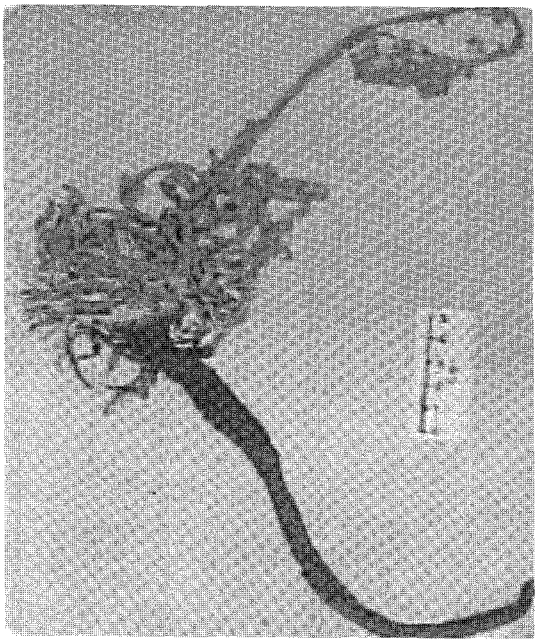


Plate 1. The dried whole plant *Arnebia nobilis.*

samples were dried at 40-50°C. The transverse sections were made for microscopical details and histochemical evaluation of various contents present in root was studied. The behaviour of powdered drugs was also studied with different chemical reagent following the methods described by Chase and Pratt (1949) and Kokoski *et al.,* (1958).

The total ash, acid insoluble and water soluble ash, alcohol and water soluble extractive contents was assayed by the I. P. methods (Anonymous, 1966) and the total phenolics, tannins (H_2S method) and sugar (Shaffer's Somogyi method) by the methods of feigl (1966) and Anonymous (1984) respectively. The total naphthaquinone content was determined by the colorimetric method at λ-516 nm by preparing a standard curve of Arnebin-1.

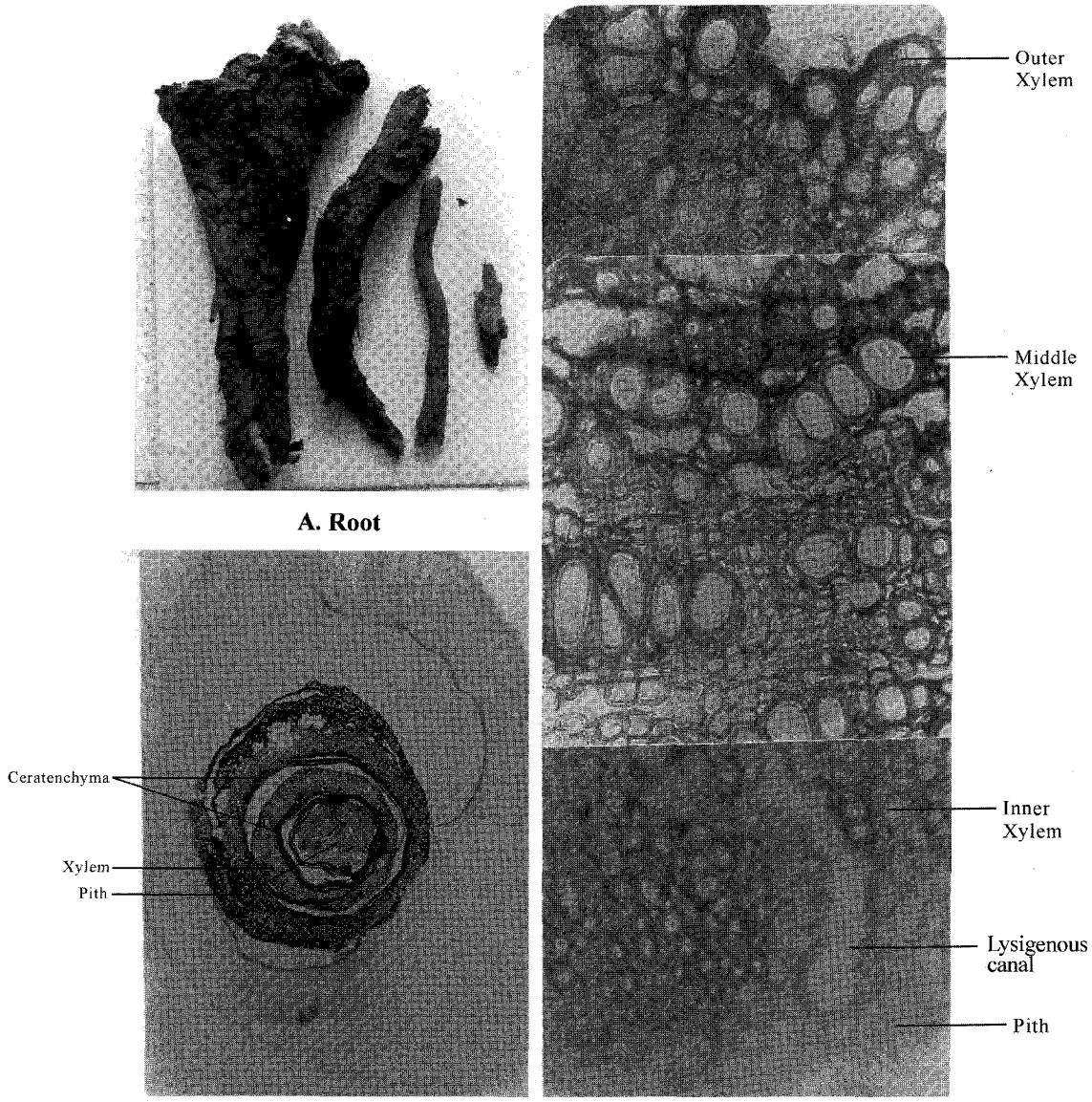
For TLC fingerprint profile, the extract was prepared with ethanol and was then fractionated with hexane. 1 mg each of arnebin-1, arnebin-3 and arnebin-4 were dissolved in 1 ml of hexane separately. Hexane soluble fractions and the solutions of arnebins were applied with a Camag Linomat IV applicator on a precoated silica gel G60 F254 TLC plates (E. merck) of uniform thickness of 0.2 mm. The finger print profiles were obtained with a Desaga Video Documentation Unit III and the amount of arnebins were determined using a Camag Densitometer Scanner with a software Cats 3.

Macroscopic characters – The crown of the dried root is tapering 1-5 cm in diameter, fusiform, often twisted. Externally the root is often furrowed, sometimes so deeply that the cylindrical form of the root is easily lost and segments are irregularly formed. The layers of bark including wood in cases readily exfoliate in the form of dried papery layers that are reddish purple in colour. The fracture of root is short and the texture is modestly coarse. The transverse section shows a semi-ring-porous wood and distinct zonations surrounded by decomposed and separated bark layers. Secondary phloem and xylem, surround the internal reddish purple zone of decomposed xylem and pith (Plate 2 A & B).

Microscopic characters – In transverse section of the root, the cortex is 25-30 cells broad with irregularly shaped parenchymatous cells. Some layers of the outer cortex become suberized and crushed, showing a brown colour with Schultge's solution. The cambium is distinct and of 3-4 cells thick and wavy in outline. The secondary phloem is 15-20 cells broad and comprises sieve tubes; compound sieve plates, companion cells and parenchyma. Alternate zones of ceratenchyma and xylem are observed. The number of vessels varies from 500 to 700 per mm^2. The outermost layer of xylem vessels consists of the clusters of broad vessels while the innermost of narrow ones. The middle portion of the xylem has broad and narrow vessels in the form of multiple clusters and sometimes in the form of alternate rings of both types of vessels. The narrow vessels are arranged in radial rows. The secondary wall of vessels are thickened in the spiral, Sclariform, reticulate form, and the intervascular pitting is alternate. Fibres are absent. The xylem parenchyma is paratracheal and narrowly confluent. They become suberized at maturaty. The pith is made up of large, thin walled parenchymatous cells. Lysigenous mucilaginous canals and rosette crystals of calcium oxalate are present in pith and xylem parenchyma region. (Plate 2B&C). In maceration, the size of the vessels varies from 46.2 μm to 184.8 μm in length and 15.4 μm to 138.6 μm in width. The trachieds are very few and with bordered pits.

Peelings – The transverse sections of the peeling show crushed and suberized parenchymatous cells. The macerated peelings show three types of suberized parenchymatous sheath: (i) polygonal somewhat rounded cells with reddish brown content, (ii) elongated tapering cells without any coloured content and (iii) polygonal suberized cells without any granulated reddish contents in them (Plate 3).

Powder – The powder is reddish purple. Microscopic examination shows numerous fragments of lignified xylem vessels, isolated or in groups with spiral to reticulate secondary wall thickening, some tailed vessels, tracheids with bordered pits; unlignified trichome with smooth and striated walls, saucer shaped bases with surrounding parenchymatous cells; rosette crystals of calcium oxalate; and the patches of the polygonal or somewhat rounded



A. Root

B. T.S. Root (Diagrammatic) (5x4)

C. T.S. Root (10x10)

Plate 2. External morphology and cross sections of *A. nobilis* root.

suberized parenchymatous cells, with or without dark red substances, patches of elongated colourless suberized cells. The powder becomes bluish purple when treated with 1N NaOH in methanol, orangish brown with Conc. HNO_3, and bright red with acetic acid.

Phytochemical studies – The percentage of total ash, acid insoluble ash, water soluble ash, alcohol and water soluble extractives, sugars, tannins, phenolics and naphthaquinones are found 10.746±0.021, 0.703±0.013, 0.241±0.016, 0.115± 0.005, 1.811±0.245, 1.35±0.024 and 10.5±0.02 respectively.

The TLC profile of hexane fraction is compared with arnebin-1, arnebin-3, and arnebin-4 by developing the plate

Table 1. TLC details of hexane-soluble portion of *A. nobilis* Root & Root stock

Rf. Value	At UV 254 nm	Colour of the band under visible light	% (w/w)
0.40 (Arnebin-4)	Black	Red	1.72
0.62 (Arnebin-3)	Black	Red	10.53
0.79 (Arnebin-1)	Black	Red	9.37

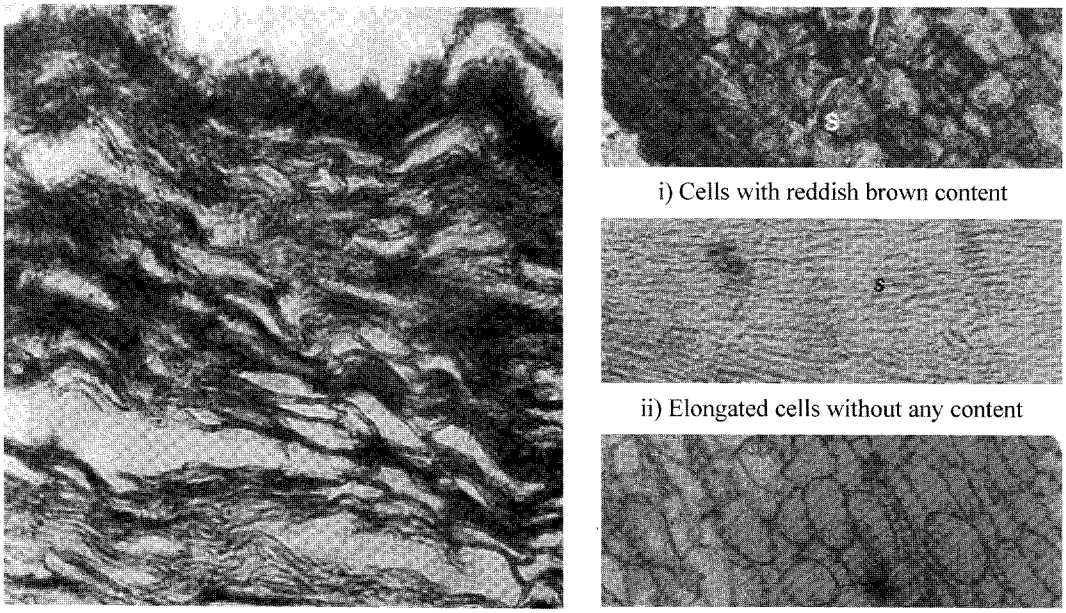


Plate 3. Peeling of *A. nobilis* root.

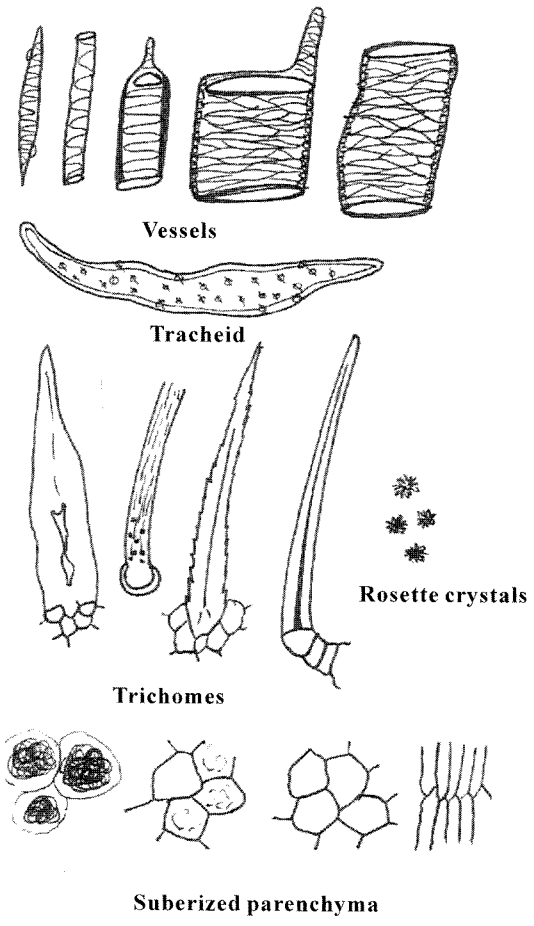


Fig. 1. Power.

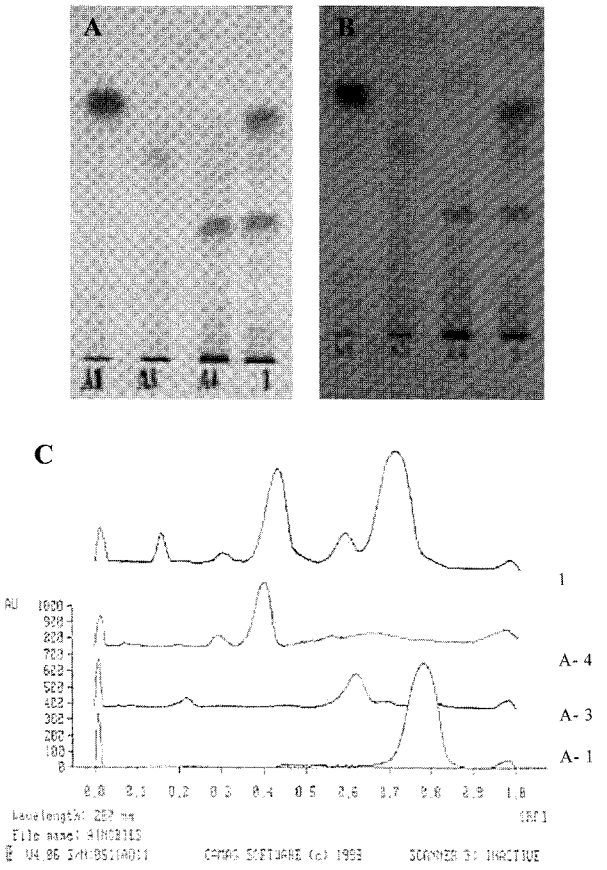


Fig. 2. TLC profile of hexane fraction of *A. nobilis* extract and reference samples A1-Arnebin 1; A3-Arnebin-3; A4- Arnebin-4; 1-Hexane fraction of ethanolic extract.

in Toluene: Chloroform: Methanol (7:3:0.05). Finger print profiles are documented under UV 254 and visible light and the percentage of arnebins and Rf values are recorded by scanning the chromatogram at wavelength 280 nm (Table 1, Fig. 2).

Acknowledgements

The authors are thankful to the Directors of National Botanical Research Institute, Lucknow and Central Drug Research Institute, Lucknow for providing facilities.

References

Anonymous, *The Wealth of India. A Dictionary of Indian Raw Material and Industrial Products* (CSIR, New Delhi) **1**, 429-31 (1950).

Anonymous. Indian Pharmacopoeia, 2nd Edn. Government of India, New Delhi. (1966).

Anonymous, *Official analysis of the association of official analytical chemists (AOAC)* 14th ed. Published by the Association of official Analytical Chemists Arlington, Virginia USA. 581-82 (1984).

Bhakuni, D.S., Dhar, M.L., Dhawan, B.N. and Mehrotra, B. N. Screening of Indian plants for biological activity: part-2. *Indian J Exp Bio* **7**, 250-62 (1969).

Bole, P.V. Botanical identity of Ratanjot. *J. Sci. Industr. Res.* **20**(C), 188-190 (1961).

Bole, P.V. Botanical identity of Ratanjot. *J. Sci. Industr. Res.* **21**(C), 354-356 (1962).

Chase, C.R., Pratt, R.J. Fluorescence of powdered vegetable drugs under ultra violet radiation. *J. Amer. Pharmacol. Assoc.* **38**, 324-331 (1949).

Feigl, F. *Spot Test in Organic Analysis* Seventh English Edition. Elsevier Publishing Company, Amsterdam, London, New York (1966).

Gupta, S.K., Mathur, I.S. Effects of *Arnebia nobilis* and its naphthaquinones in rat walker carcinosarcoma. *Indian J. Cancer* **9**, 50-55 (1972).

Katti, S.B., Shukla, Y.N., Tondon, J.S. Arnebin derivatives for anti-cancer activity. *Indian J. Chem* **18B**, 440-42 (1979).

Khatoon, S., Mehrotra, S., Shome, U., Mehrotra, B.N. Analysis of commercial Ratanjot by TLC fluorescence fingerprinting. *Int. J. Pharmacog.* **31**(4), 269-277 (1993).

Khatoon, S, Mehrotra, S. Naphthaquinoes from some Boraginaceous Taxa A Chemical Review. *Nat. Prod. Sci.* **2**(2), 75-85 (1996).

Kokoski, J., Kokoski, R., Slama, F. J. Fluorescence of powdered vegetable drug under ultra violet radiation. *J. Amer. Pharmacol. Assoc.* **47**, 715 (1958).

Papageorgiou, V. P. Naturally occurring isohexenyl naphthazarin pigments; A new class of drugs. *Planta Medica* **38**, 193-03 (1980).

Patel, R.P., Patel, R.N. Anti-microbial activity of Ratanjot The roots of *Arnebia nobilis. Indian J. Pharm* **28**: 302-04 (1966).

Shukla, Y.N., Tondon J.S., Bhakuni, D.S., Dhar, M.M. Chemical constituents of antibiotic fraction of *Arnebia nobilis. Experientia* **25**, 357-58 (1969).

(Accepted December 5, 2003)