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## Conservation and utilization of *Arnebia benthamii* (Wall. ex G. Don) Johnston – a high value Himalayan medicinal plant

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The investigation on conservation and utilization of *Arnebia benthamii* (Wall. ex G. Don) Johnston was carried out to identify optimum stage of the collection of propagules, improve upon the rooting of root cuttings and identification of optimum conditions for seedling survival. Individuals at reproductive maturity were found suitable for collection of propagules because of the occurrence of 3–5 buds at the terminal growing end of the root. These buds can be effectively utilized for vegetative propagation. Chilling for 40 days significantly ( $P < 0.05$ ) improved rooting of root cuttings. Seedling survival and growth performance were significantly ( $P < 0.05$ ) higher at a high-altitude village Lata, thereby facilitating the establishment of herbal gardens in the vicinity of natural population. This activity will not only reduce pressure on the natural population, but also has the potential to generate rural economy. Further, the possibilities of revegetating the degraded natural habitats and creating nursery centres at low-altitude areas are discussed. This study will help in developing conservation strategy for optimum utilization of *A. benthamii*.

CONSERVATION of medicinal plants is receiving increased attention all over the globe in view of their ero-

sion, and resurgence of interest in herbal medicines for health care<sup>1,2</sup>. World trade figures suggest that India ranks next to China in export of medicinal raw material (32,600 tonnes, US\$ 46 million)<sup>3</sup>. In India, large quantities of medicinal plants are extracted from the wild to meet the increasing demand of raw material for domestic consumption and export. As a result, natural habitats are depleting at a fast pace. Besides, unscientific methods of extraction and adulteration have further accentuated this complex problem<sup>4,5</sup>.

*Arnebia benthamii* (Wall. ex G. Don) Johnston [Syn *Macrotomia benthamii* (Wall.) DC.] (family Boraginaceae), a high-value Himalayan medicinal plant, ranks second in the list of medicinal plants prioritized for western Himalaya and also figures among the 59 medicinal plants prioritized for conservation<sup>4</sup> due to high extinction threat.

It is an erect, herbaceous perennial, 30–90 cm in height, occurring in the alpine and subalpine Himalaya at altitude of 3000–3900 m asl. The species is a major ingredient of the commercial drug available under the name Gaozaban, which has antibacterial, antifungal, anti-inflammatory and wound-healing properties. The roots yield a red pigment, Shikonin (a dye), which has several medicinal properties and is marketed under the trade name Ratanjot<sup>6</sup>. The species also possess stimulant, tonic, diuretic and expectorant properties. The flowering shoots are used in preparation of sherbet (syrup) and jam useful in various diseases of tongue, throat, fever and cardiac disorders. Secondary metabolites, Arnebin 1 and Arnebin 3 obtained from the other species of this genus are reported to possess anticancerous property<sup>7</sup>. *Arnebia euchroma* exhibits potent anti-HIV activity<sup>8</sup>.

As a result of over exploitation of its rhizomes for medicinal purposes, *Arnebia benthamii* has been listed in the Indian Red Data Book<sup>9</sup>. Apart from the reports on

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taxonomic descriptions, general distribution and uses; detailed investigations have not been undertaken so far, especially with regard to the development of propagation protocols and other related aspects such as *in situ* conservation, habitat management and life-history studies.

The present paper attempts to (i) identify optimum stage of the collection of propagules, (ii) improve upon the rooting of root cuttings, and (iii) identify optimum conditions for seedling survival.

The results of the investigation will help in developing strategies for conservation and sustainable utilization of the species. The identification of optimum stage for propagation and consumption of species will help in checking destructive harvesting of individuals in different stages, cultivation of the species will reduce pressure on natural population and revegetating the degraded habitats will help in restoring the population and in conservation of the species *in situ*.

The study was carried out over a period of two years (1999–2001) in Uttarakhand Himalaya. Individuals were collected (October 2000) after seed maturity. Seeds were collected, air-dried and stored in brown-paper bags at room temperature. The rootstock was brought along with the rhizosphere zone soil in plastic bags and planted with the soil in nursery till they were utilized for propagation studies.

For the rooting experiment, the entire root was dug out from the nursery, washed thoroughly with tap water and kept at 4°C in nylon-mesh bags for different time durations (10, 20, 40 d). Nylon-mesh bags were used to provide good aeration. Treatments were selected on the basis of the response of preliminary trials conducted earlier (1999). Five centimetre long terminal growing point of the root with a bud was vertically sliced and planted (October 2001) in plastic pots containing a mixture of soil and sand (2:1). The remaining basal part of the root was non-regenerative and hence excluded.

All the experiments were carried out in completely randomized block design. In each treatment, eight cuttings with three replicates were used and placed in 75% green agro shading net (Rajdeep Agri Products, New Delhi). Watering was done twice a week depending upon the requirement. After 40 days of planting, root cuttings of all treatments were uprooted and observations were made on percentage of cuttings rooted, number of roots and root length.

For seed germination, three replicates of 300 seeds were sown (March 2001) in earthen pots containing a mixture of soil and sand (2:1). The pots were placed in open condition. Watering was done every alternate day or according to requirement. Seeds were considered germinated upon appearance of cotyledonary leaves. Date of onset and completion of germination was recorded. Per cent germination was calculated on its completion.

Germinated seedlings were used for identifying optimum conditions for survival and growth performance. Three replicates of thirty-five seedlings were planted after 40 days of germination at three different altitudes: Katarmal (1240 m), Lata (2220 m) and Latakharak (3700 m). At Katarmal and Lata watering was done twice a week; however, at Latakharak (natural population) the seedlings were not watered. Survival percentage and growth performance were monitored at monthly intervals until senescence (October).

Observations of the root morphology reveal that individuals at maturity (reproductive) stage are optimum for multiplication as maximum number of buds (3–5) is found at this stage, compared to the vegetative phase with only 1–3 buds per individual.

Treated cuttings planted in plastic pots showed 75% rooting in response to chilling for 40 days, which was significant ( $P < 0.05$ ) improvement over control and other chilling pretreatments. ANOVA revealed significant ( $P < 0.01$ ) effect of treatments on percentage rooting, root length and root number (Table 1).

Seeds started to germinate after 17 days of sowing and the germination was completed within 28 days. Thirty-five per cent seeds germinated.

Seedlings transplanted at different altitudes showed variation in their survival percentage. The maximum

**Table 1.** Effect of different pretreatments on root cutting of *Arnebia benthamii*

Treatment	Per cent rooting	Root number	Root length (cm)
Control	41.7	9.3	4.9
Chilling 10 d	45.8	11.5	8.0
20 d	70.8	11.8	7.9
40 d	75.0	12.9	11.0
LSD ( $P < 0.05$ )	17.2	2.2	2.2
<i>F</i>	11.623	6.012	32.713

**Table 2.** Growth performance of *Arnebia benthamii* seedlings at different altitudes

Site	Altitude (m)	Survival (%)	Rosette diameter (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)
Katarmal	1240	60.0	11.6	4.8	5.0	2.2
Lata	2220	66.7	17.0	7.6	8.0	2.5
Latakharak	3700	20.0	4.8	4.4	3.6	1.5
LSD ( $P < 0.05$ )	–	3.42	0.59	0.52	0.72	0.29
<i>F</i>	–	105.561	112.166	32.571	152.733	22.764

seedlings survived at Lata (67%), which was significantly ( $P < 0.05$ ) higher than Katarmal (60%) and Lataharak (20%). Growth performance in relation to leaf number, leaf length, leaf width and rosette diameter was also significantly ( $P < 0.05$ ) higher at Lata as compared to other sites (Table 2).

*A. benthamii* is a monocarpic perennial and reaches reproductive maturity in 3–4 years. Thereafter, in the following years it remains in vegetative phase till it attains reproductive maturity once again (Figure 1). Individuals at reproductive phase are best suited for collection of propagules for multiplication of the species through root cuttings and seeds. As the plant completes its life cycle, the seed set thus produced in the reproductive phase ensures the occurrence of natural variation. Terminal growing point of roots consists of maximum number of buds (3–5) at this stage. This might be due to the fact that resource allocation is maximum towards the root during senescence upon inactive phase of vegetative growth<sup>10</sup>. Terminal growing point can be utilized for multiplication through vegetative propagation and seeds can be used for raising seedlings. Further, the basal part of the root, leaves, and flowering stalk can be utilized for consumption and for trade. In case the roots are not harvested at this stage the invaluable raw material is lost, because in the subsequent years the root starts decaying from the core<sup>11</sup>. Also at this stage, the active principle content is expected to be optimum as the resource allocation is concentrated towards the root.

On the contrary, if individuals are harvested at vegetative phase they do not fulfil the requirement of multiplication and utilization because (i) only 1–3 buds are present and harvesting at this stage will be destructive as the life cycle of the plant is not complete and hence no seeds are produced; (ii) invaluable germplasm and the chances of variability due to sexual reproduction are lost, and (iii) utilization of the species for consumption or trade is also not optimum. The collection of immature and underdeveloped parts results in products of inferior quality because of poor active principle content<sup>12</sup>.

Chilling pretreatment of 40 days enhanced rooting percentage (75%) in comparison to control. This is due to the fact that the roots of *A. benthamii* in natural conditions are exposed to sub-zero temperatures during winter. However, physiological reasons involved are not known<sup>13</sup>.

Relatively better survival percentage and growth performance at the high altitude village is an indication that the species can be grown by villagers in the vicinity of the natural habitat of the population. Earlier studies on high-altitude medicinal plants like *Podophyllum hexandrum*, *Aconitum heterophyllum* and *Aconitum balfourii* suggest that the cultivation in the vicinity of the natural population does not bring marked variation in the active principle of the species<sup>14,15</sup>.

Poor growth performance and survival percentage of seedlings transplanted in the natural population may be

due to the problem in acclimatization of the species raised at low altitude and transferred to higher altitude. In spite of poor survival percentage (20%) of the individuals transferred to natural habitats, the activity can help in promoting *in situ* conservation, considering that the species has been classified as critically endangered<sup>16</sup>. Further, the survival percentage can be increased if the seedlings are transferred to the natural population after one year of growth at high altitude, since by this time the root system will be well developed. Transfer of seedlings to its natural habitat will not only help in improving the status<sup>17</sup> of the population but also ensure the biosynthesis of active principle.

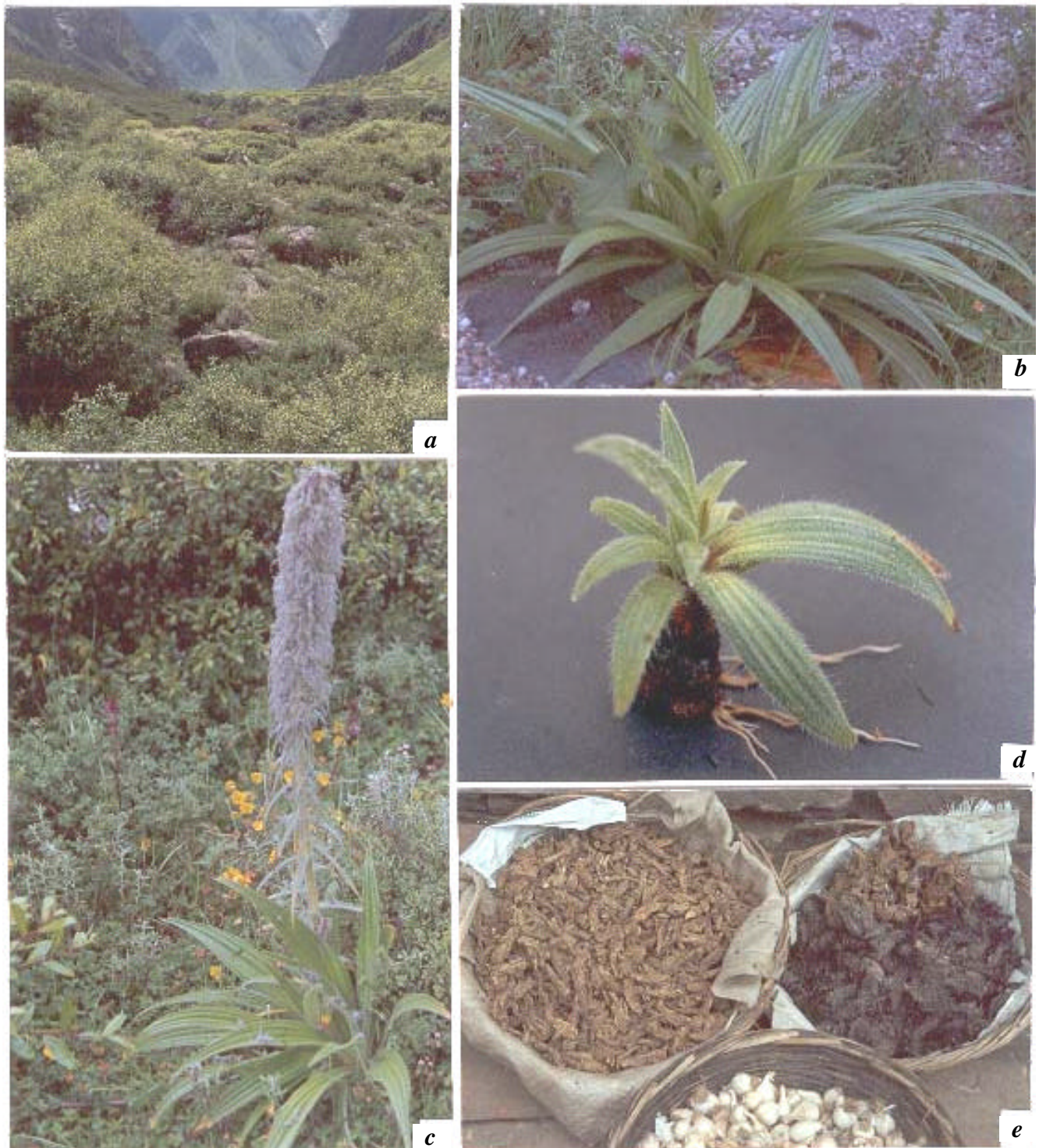
Though the growth performance and survival percentage of the species at low altitude of 1220 m asl (Katarmal) are next to Lata, it needs to be tested whether the species retains the active principle content at an optimum level. Owing to better climatic conditions, i.e. early initiation of summer, low-altitude areas can serve as nursery centres for providing propagation material to inhabitants of high-altitude villages. By raising the propagation material earlier at low altitude, plantlets will get a longer duration of growing period, which will result in better growth of the individuals resulting in greater biomass and hence greater returns.

Individuals raised through vegetative propagation from different sites can also be utilized for revegetating the wild population. Introduction of the individuals from different locations will be helpful in retaining variability and conserving genetic resources. Chances of survival and growth performance of vegetatively propagated individuals are better as they have a well-developed root system. Efforts for reintroduction of the species through root cuttings are in progress.

The outcome of the present study can be gainfully utilized for multiplication of the species, creating herbal gardens in high-altitude villages, revegetating the degraded natural populations through transplanting seedlings raised at high-altitude villages and sustainable utilization of the species. These steps will not only help in boosting the rural economy but also reduce pressure on the natural population.

Considering the economic potential of the species, cost-effective technique, and use of locally available resources, the technology has immense potential of easy adoption by the farmers. The technique can be adequately adopted by the end-users on demonstration in the farmer's field. As the species in nature occurs on sandy, rocky and moist grassy slopes, cultivation of the species can be practised on the gentle slopes normally found in the high-altitude zone. At lower altitude, propagules can be raised on gentle slopes or even flat land with proper drainage, so that the developing rootstock is not decayed due to stagnant water.

On the basis of the present investigation, it is recommended that: (i) Individuals should be harvested only



**Figure 1.** *a*, *Arnebia benthamii* habitat; *b*, Individual in vegetative phase; *c*, Reproductive phase; *d*, Vegetative propagation and *e*, Raw material for trade.

after seed set. (ii) Terminal growing point should be utilized for vegetative propagation. However, basal part of the root, flowering stalk and leaves can be utilized for consumption and trade. (iii) Chilling pretreatment is rec-

ommended for enhancing rooting percentage of root cuttings. (iv) Cultivation of *A. benthamii* can be practised in high-altitude villages. (v) Nursery centres can be created at low altitude for providing planting material to the rural

inhabitants at high altitude. (vi) Individuals raised at high-altitude villages can be utilized for revegetating the wild population, consumption and trade.

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## Interrelationship between lipid peroxidation, ascorbic acid and superoxide dismutase in coronary artery disease

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**With the current understanding on the mechanism of LDL oxidation and oxidized LDL in coronary artery disease (CAD), a pilot study was conducted to evaluate the interrelationship between lipid peroxidation, ascorbic acid and superoxide dismutase in patients suffering from CAD. Plasma malondialdehyde – a marker of lipid peroxidation was significantly elevated and the levels of ascorbic acid and superoxide dismutase were significantly reduced in patients. This scenario suggests that higher oxidant stress and reduced antioxidant status along with hypercholesterolemia and hypertriglyceridemia are the key factors for progression of atherosclerosis and hence, a management strategy aiming at simultaneous reduction of cholesterol and control of lipid peroxidation in CAD is envisaged.**

CORONARY artery disease (CAD) is the single most important disease entity in terms of both mortality and morbidity in the entire world population. Both men and

women between the age group 40 and 60 are susceptible to it. Despite all-round efforts in the prevention and management of this disease, it remains a major challenge to the health managers and scientists. It is predicted that by the year 2020 this disease would persist as the major and the most common threat to human life<sup>1</sup>. In developing countries, the incidence of CAD is increasing alarmingly. India is on the verge of a cardiovascular epidemic! By the year 2015, cardiovascular mortality is likely to rise to the order of 103% in males and 90% in females. The circulatory system disorders are going to be the greatest killer in India by the end of the year 2015 (ref. 2). The underlying cause of CAD is atherosclerosis – a disease involving a complex array of circulating blood proteins, lipoproteins and cells, and their interaction with the cells and matrix proteins of the arterial wall. It is well established that high circulatory serum cholesterol, low density lipoprotein cholesterol (LDL-C) and low levels of circulating high density lipoprotein cholesterol (HDL-C) are the main causatives of this disease. Basic research has provided strong evidence that oxidation of LDL also plays an important role in the pathogenesis of atherosclerosis. Oxidative modification of LDL is brought about by free radicals, which cause degradation of polyunsaturated fatty acids and the formation of lysolecithin, oxysterols and aldehyde modification of lysine residues on Apo B (ref. 3). Lipid peroxidation is the most studied biologically relevant, free-radical chain reaction. Cells have a comprehensive array of antioxidant defence mechanisms to reduce free radical formation or limit their damaging effects. These include enzymes such as superoxide dismutase (SOD) and catalase to degrade superoxide and peroxides respectively, and essential radical scavengers

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