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A mini review on *in vitro* propagation of *Swertia chirayita* an endangered medicinal plant

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ABSTRACT

Swertia chirayita is an endangered indigenous medicinal herb. It is used in traditional medicine to treat various ailments. *Swertia chirayita* is found in temperate regions of the Himalaya at an altitude of 1200–3000 m from Kashmir to Nepal, Bhutan and grows in the slopes of moist shady places. The species is valued for its bitterness. The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin, swerchirin, swertiamarin and other active principles of the herb. Its medicinal usage is reported in Indian pharmaceutical codex, the British and the American pharmacopoeias and in different traditional systems of medicines such as the Ayurveda, Unani and Siddha. With the passage of time there is increase in demand of this plant, so it is uprooted in its earlier stage and plant is becoming endangered. There are some biotechnological methods like *in vitro* propagation and *in vitro* conservation which can protect the medicinal plants to be extinct. These two methods help to produce maximum plants in less time and conserved the plant for long time. These are also very useful in storing valuable germplasms. This review is mainly focused on *in vitro* propagation and conservation of the *Swertia chirayita*.

KEY WORDS: *SWERTIA CHIRAYITA*, *IN VITRO* PROPAGATION, CONSERVATION, MEDICINAL PLANT, TISSUE CULTURE

INTRODUCTION

Swertia chirayita Buch.- Hams. ex Wall.belongs to family Gentianaceae. It is commonly known as "Chirata" and in sanskrit it is called as Anaryatikta, Ardhatikta, Bhunimba, Chiratika, Chiratitka, Haima, Jvarantaka, Kairata, Kandatiktaka, Kiranta, Kirataka, Kirata Tikta, Naditikta, Naipala, Nepalanimba, Nidrari, Ramasenka, Sannipatha, Sutiktaka, Trinanimba, and Viktaka (Anon, 1982 and Kritikar 1984). It is an indigenous species of temperate Himalayas. Among many species of *Swertia* in India *chirayita* is the only species which is considered most important for its medicinal properties (Joshi and Dhawan, 2005).There is no steadiness in literature that plant is either annual (Anon, 1982 and Kritikar 1984), or biennial/pluri- annual (Edwards, 1993). The plant has long been used for its blood-purifying, antifungal and antihelmintic properties (Pant *et al.* 2011). *S.*

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chirayita plants contain several active constituents such as xanthones, flavonoids, iridoids and secoiridoid glycosides that are responsible for its therapeutics properties (Kumar and Chandra, 2013).

The major phytochemicals of the bitter-tasting plant include swertiamarin, amarogentin and mangiferin, a xanthone C-glucoside (Phoboo *et al.* 2013). Swertiamarin is reported to be effective against hepatitis (Wang *et al.* 2011) and shown to exhibit anti-diabetic (Vaidya *et al.* 2013), anticancer (Kavimani and Manisenthlkumar, 2000) activities. Amarogentin is known to be anti-diabetic(Phoboo*et al.* 2013), anticancer (Pal *et al.* 2012) and anti-arthritic (Saravanan *et al.* 2014).

The plant has an erect, about 2-3 ft long stem and the whole plant is bitter in taste. It has lanceolate acute leaves with orange brown or purplish coloured stem, and contains large continuous yellowish pith. The roots are simple, tapering , stout, short and almost 7 cm long. The flowering & fruiting occurs between July to September. Flowers of *Swertia chirayita* are in the form of numerous small, axillary, opposite, lax cymes arranged as short branches small, stalked, green-yellow, tinged with purple colour, rotate and tetramerous. The corolla is twice as long as the calyx and divided near the base into four ovate–lanceolate segments. The upper surface of the petal has a pair of nectaries covered with oblong scales and ending as fringes. Fruit is a small, one-celled capsule with a transparent yellowish pericarp. It dehisces from septicidally into two valves. Seeds are numerous, minute many-sided and angular. Floral characteristics such as colourful corolla and presence of nectaries support cross-pollination in the species. *Swertia chirayita* contains a yellow bitter ophelic acid and two bitter glucosides chiratin (Joshi and Dhawan., 2005, Brahmchari *et al.*, 2004 Pant *et al.*, .2010, Chandra *et al.*, 2012, Kumar and Staden, 2016).

Swertia chirayita is difficult to propagate on mass scale via seed owing to non-availability of seeds due to harvesting of plants before seeds mature. So instead of going for conventional approaches of, the application of alternative reproducible micropropagation strategies has become inevitable for mass propagation and sustainable utilization of this age-old medicinal plant. Due to its over exploitation for different medicinal uses and commercial purposes its availability is decreasing day by day so it's becoming extinct. *S. chirayita* conservation status has been categorized as "critically endangered" (Joshi and Dhawan, 2005 and Padhan *et al.* 2015). Developing an *in vitro* regeneration protocol for *Swertia chirayita* is urgent to promote large-scale production for *ex situ* conservation and for satisfying the pharmaceutical needs. Synthetic seed technology is also an applied application of modern plant biotechnology which offers tremendous potential for easy handling, micropropagation and plant germplasm conservation through cryopreservation (Gantait *et al.* 2015 and Kumar and Staden, 2016).

This article briefly reviews the in vitro propagation and in vitro conservation of the plant. This is an attempt to compile and document information on micropropagation and in vitro conservation of *S. chirayita* and highlight the need for research.

IN VITRO PROPAGATION

Wawrosch *et al.* (1999) developed as protocol for micropropagation of *Swertia chirayita*. They found that multiplication by adventitious shoot regeneration from root explants is most suitable method for the propagation of *Swertia chirayita*. A two-step system consisting of an initial 3 weeks cultivation on modified MS medium supplemented with 3 µM 6-benzyladenine followed by another period of 3 weeks in plant growth factor free medium was used. The pH of all nutrient media was adjusted to 5.8± 0.1. The root explants taken from 6-to 8-week-old plants are very well suited for the multiplication of *Swertia chirayita* through regeneration of adventitious shoots. The explants were cultured on modified basal MS medium with 3 mMBAP for 3 weeks, followed by another 3 weeks on hormone-free basal medium. An average of 1.9 very healthy shoots per 5-mm explant were obtained. Dipping of the shoots in an aqueous solution of NAA (15 ppm) followed by 3 to 4 week cultivation period on hormone-free, half-strength MS medium proved to be the most efficient method for rooting of *Swertia chirayita*.

Chaudhuri *et al.* (2007) produced genetically uniform plants from nodal explants of *Swertia chirayita* Buch. Ham. ex Wall. Shoot regeneration was obtained in shoot inducing medium containing half-strength MS basal medium supplemented with 0.44 µM 6-BAP and 4.65 µM 6-furfurylaminopurine. The highest number of shoots, that is 18 shoots per explant were obtained when medium was again used with 10 mM KNO_3 and 75 mg/l of casein hydrolysate. The plantlets were successfully transferred to the field and produced viable seeds.

Joshi and Dhawan (2007) described the micropropagation of *Swertia chirayita* through axillary shoot multiplication from 4 weeks old seedling derived nodal explants. 4.5 fold multiplication was obtained after every 4 weeks on MS medium supplemented with 4µM BAP and 1.5µM 2ip. Rooting was optimized on modified MS medium supplemented with 1µM NAA and 500 mg of activated charcoal which showed 94% of rooting.

A protocol for plant regeneration through indirect organogenesis was established by Bisht *et al.* (2008) for *Swertia angustifolia* Buch.-Hams. Callus was induced on MS basal medium supplemented with cytokinin (Kinetin or BA) and auxin (2,4-D/IBA/NAA) from leaf, petiole and stem explants. Higher concentration of Kinetin and

2,4–D (2.5-3.0 mg/l) exhibited best callusing in leaf and better in petiole explants. BA and NAA in the range of 1.5-2mg/l exhibited fast proliferation in callus mass in both explants.Shoots were regenerated on MS medium containing BA (1.5– 2.5 mg/l) and IBA or NAA (0.5-1.5 mg/l). Rooting was obtained with full or half MS media with IBA or NAA (0.5 -1.5 mg/l).

Balaraju *et al.* (2009) established rapid system for micropropagation of *Swertia chirayita* Buch. Hams. ex Wall. using shoot tip explants derived from *in vitro* grown seedlings. MS medium containing BAP (1.0 mg/l) and Kinetin (0.1 mg/l) along with 2% sucrose induced highest number of multiple shoots per explants. Micro proliferated shoots were transferred to elongation medium amended with 0.1 mg/l GA_3. The highest frequency of rooting was obtained in half MS medium supplemented with 0.1 mg/l NAA.

Wang *et al.* (2009) investigated the effects of phyto hormones on shoot regeneration from the leaves of field grown *Swertia chirayita* .The best result obtained in MS medium supplemented with 13.2µM 6- BAP and 0.54 µM α- NAA. The highest rate of shoot regeneration was 96.5% on the medium with 0.54 µM NAA. Adventitious shoots were transferred on the rooting medium. Rooting was optimized on MS medium supplemented with NAA 5.40µM. Pant *et al.* (2010) developed an efficient protocol for *in vitro* propagation of *Swertia chirayita*. Axillary shoot bud multiplication was achieved using nodal segments as explants. A combination of BAP 4.4 µM + IAA 2.85 µM + Adenosine sulphate 271.45 µM proved to be the best giving 11.8 fold multiplication with average shoot length of 1.9 cm after 4 weeks and 18.5 fold multiplication with mean shoot length 2.6 cm was observed. Best rooting was observed on MS medium with IBA 4.90µM. Maximum mean number of root per shoot 35.3 was observed after 8 weeks.

Pant *et al.* (2011) described procedure for regeneration of complete plantlets of *Swertia chirayita* via indirect organogenesis. Callus was obtained from *in vitro* regenerated roots on MS medium supplemented with varying concentrations of BAP and 2,4-D. BAP (13.32 µM) in combination with 2,4-D (0.90 µM) proved to be the most effective concentration for callus induction, multiplication and adventitious shoot regeneration from callus surface. The optimal hormone combination for shoot multiplication was shown to be BAP (8.88 µM), IAA (2.85 µM) and 271.45 µM adenine sulphate (Ads). Individual elongated shoots were rooted on half-strength MS medium supplemented with varying concentrations of auxins. Best rooting was obtained with MS Medium supplemented with 4.90 µM IBA. *In vitro* raised plantlets with well developed shoots and roots were acclimatized successfully.

Jha *et al.* (2011) carried out *in vitro* propagation and conservation of *Swertia bimaculata* Hook. f. & Thomas. Seeds were germinated aseptically with low concentration of BA (2.22 µM) or Kinetin (2.32 µM). The best response of *in vitro* grown shoots was obtained on MS medium with BA (2.22 µM), Kinetin (2.32 µM) and NAA (0.54µM). The number of shoots were increased to 20.6 on addition of 10 mM potassium nitrate (KNO_3) in the medium. Isolated shoots induce 100% rooting on basal medium with in 5 weeks. Rooted plants were hardened and transplanted in soil with 80-90% survival rate.

CONSERVATION OF *SWERTIA CHIRAYITA*

Over exploitation of plant sources is a normal occurrence due to its increasing demand. It is mostly used as traditional drug. The demand of this plant is on rise at both national and international level due to its multiple uses which leads to increase over harvesting of wild populations and ultimately in reduction of population. According to the International Union of Conservation of Nature (IUCN) criteria, *S.chirayita* conservation status has been categorized as "critically endangered" (Joshi and Dhawan, 2005). There are limitations in the use of seed propaga-

Table 1. list of important bioactive constituents isolated from *Swertia chirayita*

Active constituents	Biological activities	References
Amarogentin (chirantin)	Topoisomerase inhibition, chemo-preventive and antileishmanial effects .	[Ray 1996), [Saha and Dass 2005), [Phoboo *et al.* 2013]
Amaroswerin	Gastro-shielding	[Niiho 2005], [Phoboo *et al.* 2013]
Gentianine	Anti-inflammatory, anesthetic, antihistaminic, anticonvulsant properties, hypotensive, antipsychotic, lenitive, diuretic, antimalarial, antiamoebic and antibacterial properties.	[Song Zhen Yu 1958; Geng Tao 1959; Kwak 2005]. [Bhattacharya 1974], [Mansoor and Malghani MAK, 2005] , [Natarajan *et al.*, 1974, [Phoboo *et al.* 2013]
Swerchirin	Antimalarial, hypoglycemic, hepatoprotective, pro-heamatopoitic, and weak chemo preventive pharmacological effects.	[Arino 1997], [Bajjpai 1991], [Saxena 1996], [Ya 1999] [Hirkawa1987], [Phoboo *et al.* 2013].
Swertiamarin	Analgesic property	[Lei 1982], [Phoboo *et al.* 2013]
xanthones, flavonoids, iridoids and secoiridoid glycosides	Therapeutic properties	Joshi and Dhawan 2005

tion, due to low viability, and low germination percentages (Badola and Pal, 2002; Chandra *et al*., 2012).

Biotechnology offers new means of conservation of *Swertia* chirayita. Synthetic seed production is one of them.In this method somatic embryos are encapsulated in a suitable matrix like sodium alginate along with insectisides, fungicides and herbicides. Kumar *et al*. (2014) reported on synthetic seed production and plant regeneration of *S.chirayita* from somatic embryos. However, studies are required to improve this technology so that it can be used on large scale.. Cryopreservation this is also one another method of conservation. In this method, the cells are preserved in the frozen state. The germplasm is stored at a very low temperature using liquid nitrogen (at-196°C). The cells stay in completely inactive state and thus can be conserved for long periods. Certain compounds like- DMSO (dimethyl sulfoxide), glycerol, ethylene, propylene, sucrose, mannose, glucose, praline, acetamide are added during the cryopreservation. These are called cryoprotectants and prevent the damage caused to cells by reducing the freezing point and super cooling point of water., (Ara *et al*., 2000; Sharma *et al*., 2013a; Perveen and Anis, 2014; Gantait *et al*., 2015)

CONCLUSION AND FUTURE PERSPECTIVES

This review article revealed the morphogenetic potential of leaves of *Swertia chirayita* as a source for micropropagation. The explants can be easily and regularly obtained from established shoot cultures and do not require disinfection treatment hence being ideal for germplasm exchange and cryopreservation. Normal leaf culture establishment for a number of plant species have the ability to accumulate secondary metabolites and plays important role in pharmaceuticals. *In vitro* conservation was done by cryo conservation. The government has imposed total ban on collection or removal of planting materials of this important species from their natural populations but this is not possible without the support of local healers. There should be awareness among local peoples to control the overexploitation. Scientists cannot conserve this species without the help of local healers. There are many institutes and universities where the research work is going on but that work should be explored at higher level so that other researchers gain experience from that and help in conserving endangered medicinal plants otherwise this species also become extinct. Biotechnological approaches are required in future also to promote its medicinal use.

REFERENCES

Anon.(1982) In The Wealth of India: Raw Materials,CSIR, New Delhi. vol. X: Pages 78–81

Ara H., Jaiswal U., and Jaiswal V.S. (2000) Synthetic seed: prospects and limitations. Current Science Vol. 78: Pages 1438–1444.

Arino A. (1997) The extraction of yellow gentian root (Gentianalutea L.) Zeitschrift fur Leben smitteluntersuchung und-Forschung, Vol.205: Pages 295-299

Badola H. K. and Pal M.(2002) Endangered medicinal plant species in Himachal Pradesh. Current Science Vol 83: Pages 797–798.

Bajpai M.B. (1991) Hypoglycemic effect of swerchirin from the hexane fraction of Swertia chirayita. Planta Me Vol. 57:Pages 102-104

Balaraju K, Agastian P. and Ignacimuthu S.(2009) Micropropagation of *Swertia chirata* Buch. Hams. ex Wall : a critically endangered medicinal herb. Acta Physiologiae PlantarumVol 31No 3 : Pages 487- 494

Bhattacharya S. K. (1974) Letter: Chemical constituents of gentianaceae. XI. Antipsychotic activity of gentianine. J Pharm Sci Vol .63: Pages 1341-1342

Bisht S. S., Bhandari S. and Bisht N. S. (2008) In vitro organogenesis in *Swertia angustifolia* . Journal of Hill Research Vol 21 No. 2: Pages 41-46

Brahmchari G., Mandal S., Gangopadhyay A., Gorai D., and Mukhopadhyay B.(2004) *Swertia* (Gentiananceae):chemical and pharmalogical aspects.Chem. Biodiversity Vol. 1:Pages 1627-1651

Chandra S., Kumar V., Bandopadhyay R.,and Sharma M. M.(2012) SEM and elemental studies of *Swertia chirayita:* a critically endangered medicinal herb of temperate Himalayas. Current Trends Biotechnology Pharm. Pages 6381–6388.

Chandra S., Kumar V., Bandopadhyay R.,and Sharma M.M.(2012) SEM and elemental studiesof *Swertia chirayita* critically endangered medicinal herb of temperate Himalayas. Curr.Trends.Biotechnol.Pharm. Pages 6381–388

Chassot P. (2000) Phylogenetic position of the genus *Swertia* (Gentiananceae) in the subtribe Swertiinae .American Journal of Botany. 87(suppl):Pages 118-119

Chaudhuri R. K., Pal A. and Jha T. B. (2007) Production of genetically uniform plants from nodal explants of *Swertia chirayita* Buch. Ham. Ex Wall: an endangered medicinal herb. In Vitro Cellular and Developmental BiologyPlantVol.43 No.5: Pages 467-472

Edwards D.M.(1993)The marketing of non-timber forest product from the Himalayas : The trade between East Nepal and India rural development Forestry Network. Rural Development For Network.Pages1-21

Gantait S., Kundu S., Ali N., and Sahu N.C.(2015)Synthetic seed production of medicinal plants:a review on influence of explants, encapsulation agent and matrix. ActaPhysiol.Plant 37,98.doi:10.1007/s11738-015-1847-2

Gaur R.D. (1999) Flora of the District Garhwal North West Himalaya (with ethnobotanical notes)Transmedia, Srinagar (Garhwal) Page208

Geng Tao. (1959)Journal of Physiology. Vol. 23:Page 203

Hirkawa K. (1987) Chemo preventive action of xanthone derivatives on photosensitized DNA damage. Photochemical Photobiology Vol .81: Pages 314-319

Jha T. B., Dafadar A. and Chaudhuri K. R. (2011) Somatic embryogenesis in *Swertia chirata* Buch. Ham. Ex Wall: a multipotent medicinal herb. Asian Journal of Biotechnology Vol 3: Pages 186-193

Joshi P and Dhawan V.(2005) *Swertia chirayita* –an overview. Current Sciences.vol.89 No. 4:Pages 635-640

Joshi P, and Dhawan V. (2007) Axillary multiplication of Swertia chirayita: a critically endangered medicinal herb of temperate Himalayas. In Vitro Cellular and Developmental Biology PlantVol. 43 No.6: Pages 631-638

Kavimani S. and Manisenthl kumar K. T. (2000) Effect of methanolic extract of *Enicostemma littorale* on Dalton's ascitic lymphoma. Journal of Ethnopharmacology Vol. 71 :Pages 349–352

Kirtikar K. R. and Basu B. D. (eds)(1984) Indian Medicinal Plants, Allahabad.vol. III: Pages 1664–1666

Kumar V. and Chandra S. (2013) Efficient regeneration and antioxidant activity of the endangered species *Swertia chirayita*. International Journal of Pharma and Bio Sciences Vol 4 No. 4:Pages 823-833

Kumar V. and Staden J. V.(2016) A Review of *Swertia chirayita* (Gentianaceae)as aTraditional Medicinal Plant. Frontiers in Pharmacology Vol. 6(308):Pages1-14

Kwak W. J. (2005) Effects of gentianine on the production of proinflammatory cytokines in male Sprague-Dawley rats treated with lipopolysaccharide (LPS) Biol Pharm Bull, Vol. 28: Pages750-753

Lei Wei Ya (1982) *Swertia marins* central inhibitory effects. Journal of Chinese Materia Medica, Vol 13: Page 368

Mansoor A and Malghani MAK. (2005) .Diuretic effect of *Gentiana olivieri* and its alkaloid gentianine .Botany. m plants. August 13-17, Austin, Texas. Oral presentation

Natarajan P. N., Wan A. S., and Zsaman V. (1974) Antimalarial, antiamobeic and toxicity tests on gentianine. Planta Med. Vol .25: Pages 258-260

Niiho Y. (2005) Gastro protective effects of bitter principles isolated from Gentian root and *Swertia* herb on experimentally induced gastric lesions in rats. Journal of Natural Medicine, Vol. 60:Page 888

Padhan J. K., Kumar V., Sood H., Singh T. R. and Chauhan R. S. (2015) Contents of therapeutic metabolites in *Swertia chirayita* correlate with the expression profiles of multiple genes in corresponding biosynthesis pathways. Phytochemistry DOI 10.1016.

Pal D., Sur S., Mandal S., Das A., Roy A. , Das S. and Panda C. K. (2012)

Prevention of liver carcinogenesis by amarogentin through modulation of G1/S cell cycle check point and induction of apoptosis. Carcinogenesis Vol.33 :Pages2424–2431

Pant M., Bisht P. and Gusain M. P. (2011) In vitro propagation through root derived callus culture of *Swertia chirata* Buch. Ham. ex Wall. African Journal of Biotechnology Vol 11 No 29 :Pages 7408-7416

Pant M., Bisht P., and Gusain M. P. (2010) In vitro propagation through axillary bud culture of *Swertia chirata* Buch. Ham. ex Wall: an endangered medicinal herb. International Journal of Integerative Biology Vol. 10 No 1:Pages 48-53

Perveen S., and Anis M. (2014) Encapsulation of internode regenerated adventitious shoot buds of Indian Sirisin alginate beads for temporary storage and two fold clonal plant production. Acta Physiology Plantareum Vol. 36, Pages 2067–2077. doi: 10.1007/s11738-014-1584-y

Phoboo S., Pinto M. D. S., Barbosa A. C. L., Sarkar D. , Bhowmik P. C., Jha P. K. and Shetty K. (2013) Phenolic-linked biochemical rationale for the anti-diabetic properties of *Swertia chirayita* (Roxb. ex Flem.) Karst. Phytotherapy Research Vol. 27 :Pages 227–235

Ray S. (1996) Amarogentin, a naturally occurring secoiridoid glycoside and a newly recognized inhibitor of topoisomerase1 from *Leishmania donovani* J Nat Prod, Vol. 59: Pages 27-29

Saha P., and Das S. B. (2005) Fraction of *Swertia chirata* prevent carcinogenic risk due to DMBA Exposure. Indian Journal of Medical Research, 22-27 Poster Presentation

Saravanan S., Hairul Islam V. I., Prakash Babu N., Pandikumar P., Thirugnana sambantham K., Chellappandian M ., Simon D. R., Gabriel C., Paulraj M. and Ignacimuthu S. (2014) *Swertia marin* attenuates inflammation mediators via modulating NF-jB/I jB and JAK2/STAT3 transcription factors in adjuvant induced arthritis. European Journal of Pharmaceutical Sciences Vol. 56: Pages70–86

Saxena A. M. (1996)Mode of action of three structurally different hypoglycemic agents: a comparative study. Indian Journal of Experimental Biology Vol. 34: Pages 351-355

Sharma N., Varshney V. K., Kala R. P., Bisht B., and Sharma M.(2013) Antioxidant capacity and total phenolic content of *Swertia chirayita* (Roxb.ex Fleming) H. Karst. In Uttarakhand. Int.J.Pharm.Sci.Rev.Res. Vol 23:Pages 259–261.

Song Zhen Yu. (1958) Journal of Physiology. Vol. 22 :Page 201

Vaidya H., Goyal R. K. and Cheema S. K. (2013) Anti-diabetic activity of swertiamarin is due to an active metabolite, gentianine, that upregulates PPAR-c gene expression in 3T3-L1 cells. Phytotherapy Research Vol. 27: Pages 624–627

Wang Li, An Lizhe, Hu Yan Ping, Wei Lixin and Li Yi. (2011) Influence of phytohormones and medium on the shoot regeneration from leaf of *Swertia chirata* Buch. Ham. ex Wall in vitro. African Journal of BiotechnologyVol 8 No 11: Pages 2513-2517

Wawrosch C., Maskay N., and Koop B. (1999) Micropropagation of the threatened Nepalese medicinal plant *Swertia chirata* Buch-Ham. Ex Wall. Plant Cell Report Vol. 18 No 12: Pages 997-1001

Ya B. Q. (1999) Protective effect of swerchirin on hematopioesis in 60 Co-irrdiated mice. Phytomedicine Vol. 6: Pages 85-88