REVIEW OF LITERATURE

2.1. SWERTIA CHIRAYITA-THE EXPERIMENTAL PLANT

Swertia Linn. (Gentianaceae – Gentianeae – Swertiinae) is a morphologically diverse but taxonomically distinct genus. The taxon in its present circumscription comprises *ca* 150 species and is annual, biennial or perennial herb ranging from 2-4 cm to over 1.5 m in height. The genus mostly occurs in alpine or temperate habitats in Asia, Africa and North America. The circumscription of the genus has often been debated, resulting in disagreement amongst taxonomists due to the morphological similarities (nectariferous and rotate corolla lobes) among the species of S*wertia* and the related genera.

Information on taxonomy, distribution, ethnobotany and conservation of the *Swertia* species are very limited (Bhattarai, 1992; Joshi, 1988, Joshi, 2000, 2004; Joshi and Joshi, 2005; Manandhar, 2002; Sacherer, 1979; Shrestha *et al*., 1998). Although several authors have tried to enumerate the species and varieties, systematic classification of *Swertia* has still been problematic. Four problems still need to be solved: species delimitation, section delimitation, and relation with allied genera and domestication of the species for economic benefits.

2.1.1. Origin

Swertia sp. (Family Gentianaceae), included in the endangered medicinal plants list, has been used in the preparation of Ayurvedic medicine since ancient times. The plant is a native of North India, growing in the mountainous districts and has been held in considerable esteem as a medicine by the Hindus. About 150 species of the plant have been recorded from the different corners of the world. In India, there are 40, in Nepal 30, in China 75 and in Bhutan there are 23 different species. According to a recent research done on the plant collected from eleven countries of the world, Lina Strew, Assistant Professor of Rutgrus University, America has unveiled the secret that in the Himalayan regions of South and West China lays the origin of chirata. It is believed that the source of origin of *Swertia* found in Eastern Himalaya is also from the same place (S Pant, 2007).

2.1.2. Taxonomy: Checklist of *Swertia* sp.

The major contributions relating to the species of *Swertia* found in Eastern Himalayas were made by national, regional and international botanists and institutions. Among the contributions on documentation of the taxa, the significant collections and studies of the species of *Swertia* date back to Smith (1970), who had described three new species: *Swertia acaulis,* collected on open slopes at 4600m; *S. gracilescens,* collected on grass slopes at 3700m; and *S. staintonii,* collected on open slopes at 3800m. In 1976, 21 species of *Swertia* found in Eastern Himalaya have been documented in *Catalogue of Nepalese Vascular Plants* (Malla *et al.*, 1976a) which include *S. acaulis, S. alata, S. angustifolia, S. bimaculata, S. chirayita, S. cordata, S. cuneata, S. dilatata, S. gracilescens, S. hispidicalyx, S. hookeri, S. kingii, S. multicaulis, S. nervosa, S. paniculata, S. pedicellata, S. petiolata, S. purpurascens, S. racemosa, S. speciosa,* and *S. tetragona.* Similarly, Hara *et al.* (1982) in *Enumeration of Flowering Plants of Nepal Vol. III* enumerated 27 species of *Swertia* along with bibliographic citation and synonyms.

In 1984, Polunin and Stainton described 8 species of *Swertia* occurring in Nepal: *S. angustifolia, S. alternifolia, S. cuneata, S. hookeri, S. multicaulis, S. petiolata, S. racemosa, S. speciosa* in their book *Flowers of the Himalaya*. Ohba and Akiyama (1992) have reported 7 species of *Swertia*: *S. acaulis, S. cuneata, S. dilatata, S. hookeri, S. macrosperma, S. multicaulis,* and *S. pseudo-hookeri* from the alpine areas of the Eastern Nepal. In 2000, *Annotated Checklist of the Flowering Plants of Nepal* was published that includes 28 species and 4 varieties of *Swertia* (Press *et al.*, 2000). *Swertia gracilescens,* which was earlier reported as new species by Smith in 1970, is removed and has been assigned as the synonym of *Swertia paniculata.* The *Flowering Plants of Nepal* (Phanerogams), (edited by Bista, Adhikari and Rajbhandari, 2001) enumerated 29 species with 3 varieties of *Swertia.* In this categorization, both *S. gracilescens* and *S. paniculata* have been kept as distinct species and *S. dilatata* without varieties. Shah (1990, 1992) and Chassot (2003) have reported new species: *Swertia nepalensis* and *S. barunensis* from Eastern Himalaya respectively. *S. wardii* has been documented from the foothills of the Kanchenjunga Mountain, Eastern Nepal (WWF, 2008).

The circumscription of *Swertia* has been subject to major change ever since its establishment. The identification and systematic arrangement of species have been carried out based on only morphological characteristics. These works show controversy in species

and varieties boundaries. Some species are excluded in the respective groups and some are placed as synonyms and some new species added to the family.

Table 1.4: List of species of *Swertia* and their geographical distribution in Darjeeling and Sikkim Hills.

NAME	DISTRIBUTION	ELEVATION (in feet)
Swertia tonglensis Burkill	Tonglo	9,000
Swertia chirayita Roxb. ex Fleming (Karsten)	Senchal	8,000
Swertia purpurascens Wall.	Jorepokhri	3,000
Swertia cordata Wall.	Darjeeling	12,000
Swertia bimaculata Hk.f.	Simana Busty	8,000
Swertia dilatata Clarke	Senchal	8,000
Swertia rex Clarke	Singalila	11,000
Swertia hookeri Clarke	Tshango	12,000
Swertia burkiliana Smith	Tshango	12,000
Swertia racemosa Smith	Karbonang	9,000-10,000
Swertia tibetica King	Yalung	12,000
Swertia cuneata Wall.	Giagong	15,000
Swertia multicaulis Don	Paigu	13,000
Swertia nervosa Wall.	Karbonang	9,000
Swertia kingii Hk.f.	Tari and Tshango	13,000

Swertia shows wide range of morphological variation within and among the population resulting in considerable uncertainty about the delimitation of species. Among the collected plant specimens from different areas, *S. racemosa* shows a pronounced variation in the presence of cilia on margin of leaf, sepals, and bracts as well as on veins. Similarly wide ranges of variation in morphological traits among the population of *S. chirayita* and *S. nervosa* were also reported by Raskoti and Sakya (2004) and Pant and Bimb (2005) respectively.

2.1.3. Ethnobotanical value of *Swertia* sp.

The works relating to the ethnobotanical investigation and sustainable management of the resources including *Swertia* species are very sporadic (Baral and Kurmi 2005; Bhattarai, 1992; Jha *et al.*, 2001; Joshi, 1988; Manandhar, 2002; Shrestha, 1991). Joshi

(2008) has documented the medicinal uses of some species of *Swertia* (Table 1.5). Some works on the ethnomedicinal uses of *S. alata, S. bimaculata, S. cuneata, S. kingii, S. tetragona* have also been carried out (Ghimire, 2001; Manandhar, 2002; Sacherer, 1979). Among the species, *S. chirayita* is the most important for its medicinal properties. Herbal medicines such as Diabecon, Melicon V-ointment, Ayush-64 and Mensturyl syrup contain chirata (*Swertia chirayita*) extract in different amounts for its antipyretic, hypoglycaemic, antifungal and antibacterial properties (Joshi and Dhawan, 2005).

Table 1.5: Ethnomedicinal uses of some species of *Swertia* (Source- Joshi, 2008)

BOTANICAL NAME	USES
Swertia angustifolia Buch.-Ham. ex D. Don	Plant is crushed and boiled in water and two teaspoonful decoctions is given to treat malaria fever 2-3 times a day; root juice is taken to give relief from cold and cough.
Swertia chirayita (Roxb. ex Fleming) Karsten	The plants are dipped in water overnight and the bitter juice is taken the next morning to cure malarial fever; decoction of the plant is used as a tonic that influences the digestive organs and also used as antihelmintic, especially for children. Juice of the root is taken to cure liver diseases; paste of the plant is used in common ailments like cough, cold, asthma, headache and fever; roots crushed and paste rubbed over joints for quick relief; leaves warmed and paste prepared with mustard oil applied over boils and scabies.
Swertia ciliata (D. Don ex G. Don) B.L. Burtt	Decoction of plant is given 3 times a day for 5-7 days to control cough, cold and fever. It is also used as a substitute for *S. chirayita.*
Swertia delatata C.B. Clarke	Paste is applied locally to get relief from joint pains; extract is used to treat scabies; juice of plant is taken orally twice a day before meal to treat fever and headache.
Swertia multicaulis D. Don	Plant ground and paste applied over wounds for healing; 2-3 teaspoonful of plant decoction given twice a day to cure fever, cough and cold; plant decoction is also given for 2-3 days as antihelmintic.
Swertia nervosa (Will. ex G. Don) C.B. Clarke	Decoction of root is applied in skin diseases; plant is crushed and boiled in water and two teaspoonful decoctions is given twice a day in an empty stomach to treat malaria fever; extract of the plant is also given in the morning to cure gas ball and stomach problems.
Swertia paniculata Wall.	Decoction of the plant is used as a tonic; also used as a substitute of *S. chirayita* in the treatment of malarial and other fever.
Swertia pedicellata Banerji	Plant paste is applied externally on forehead to get relief from headache.
Swertia racemosa (Wall. ex Griseb.) C.B. Clarke	Plant is tonic; two teaspoonful of decoction of plant is given twice a day to treat fever and cough; paste of the plant is applied locally to treat eczema and pimples; juice of aerial part is taken orally twice a day before meals to treat jaundice.

2.1.4. Systematic position of *Swertia chirayita*

Class – Angiospermae
Order – Gentianae
Family – Gentianaceae
Genus – *Swertia*
Species – *S. chirayita*
Scientific name – *Swertia chirayita*

2.1.5. Nomenclature and Systematics

Swertia chirayita (Roxb. ex Fleming) H. Karsten, commonly known as clearing nut tree, bitter stick, Indian chiretta, Indian gentian, is also mentioned in the literature as *Swertia chirata* Buch.-Ham.; *Ophelia chirata* Grisebach; *Agathotes chirayita* Don, *Gentiana chirayita* Roxburgh (Anon, 1982; Kirtikar and Basu, 1984 and Clarke, 1885) and *Gentiana floribunda* Don (Clarke, 1885). *Swertia* is named in honour of Emanuel Swert, a Dutch gardener and illustrator. It is known by an array of names, suggesting its widespread use. Chirata is called Anaryatikta, Ardhatikta, Bhunimba, Chiratika, Chiratitka, Kairata, Kirataka, Kirata Tikta, Naditikta, Naipala, Nidrari, Sutiktaka, Trinanimba, and Viktaka (Anon, 1982; Kirtikar and Basu, 1984) in Sanskrit, Cherayata in Patna, Chirrato and Chiraita in Nepal, Chiraita and in Mumbai, Chirayatin in Gujarat, Chireta in Bengal, Nilaveppa in Kerala, and Sekhagi in Burma. It is also called Chiaravata (Urdu); Qasabuzzarirah (Arab, Farsi); Nelabevu (Kannada); Nenilawandi, Nilavembu, Shirattakuchi (Tamil). The trade name of *S. chirayita* is chiretta (Anon, 1982; Kirtikar and Basu, 1984).

2.1.6. Distribution

Chirata is found everywhere in the world, except in South America and Australia. The plant is a native of temperate Himalayas, found at an altitude of 1200-3000m (4000 to 10000 ft), from Kashmir to Bhutan, and in the Khasi Hills at 1200-1500m (4000 to 5000 ft) (Kirtikar and Basu, 1984; Clarke *et al.,* 1885). It can be grown in sub-temperate regions between 1500 and 2100m altitudes (Bentley and Trimen, 1880). Plants are found to grow wild at high altitude (6000 to 10000 ft.) in the small pockets of Darjeeling, Kumaoun and Chotonagpur Hills of India. Some species of *Swertia* have also been reported in Nepal, Japan, and China.

2.1.7. Ecology-variation

Little research has been done to identify the existing diversity among different populations of *S. chirayita*. Considering the range of different niches occupied by the plant, there is a possibility that many ecotypes and/or chemotypes of *S. chirayita* exist. It would be interesting to study the morphological, molecular and biochemical variations among different populations for *S. chirayita.* Cytological work done on the species is poor. Khoshoo and Tandon (1963) used pollen-mother cells for cytological studies in some Himalayan species of *Swertia*. The authors counted thirteen bivalents at metaphase I, and observed that one of them was bigger than the rest. Molecular investigation was undertaken to understand the level of genetic diversity in five *S. chirayita* populations of Nepal using Polymerase Chain Reaction (PCR)-based Random amplified polymorphic DNA (RAPD) technique. Among the five populations investigated, the mean genetic similarity among the *S. chirayita* populations varied from 33% to 68%. The high genetic polymorphism reflected in *S. chirayita* populations indicates the good survival potentiality and adaptability in changing environmental scenario. The results thus produced might be helpful to plant breeders for elite cultivar development (Shrestha *et al.*, 2013).

2.1.8. Morphology

2.1.8.1. Macromorphology

The habit of chirata is ambiguous in literature. Some authors have described it as an annual (Anon, 1982; Kirtikar and Basu, 1984) and others as biennial or pluri-annual (Edwards, 1993). It is not clear whether the plant behaves differently due to climatic conditions or varying genotypes. The plant can be grown in a variety of soils with sandy loam rich in carbon and humus. It is also found in open ground and recently slash-and-burnt forests (Edwards, 1993). It has an erect, about 2-3 ft long stem, the middle portion is round, while the upper is four- angled, with a prominent decurent line at each angle. The stems are orange brown (Anon, 1982) or purplish in colour (Bentley and Trimen, 1880), and contain large continuous yellowish pith. The leaves are lanceolate, or ovate-acuminate, and cordate at the base, smooth, entire, very acute, sessile, clasping, and marked with 3, 5 or 7 nerves and its length ranges from 8 to 9 cm. The root is simple, tapering and stout, short, almost 7 cm long and usually half an inch thick (Clarke, 1885 and Bentley and Trimen, 1880).

Flowering in *S. chirayita* is in the form of numerous small, axillary, opposite, lax cymes arranged as short branches and the whole inflorescence is 2 ft long. It can reach up to 1.5 meters high. Flowers are small, stalked, green-yellow, tinged with purple colour, rotate and tetramerous (Kirtikar and Basu, 1984; Bentley and Trimen, 1880). 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 (Bentley and Trimen, 1880). The stamens are 4, style single, and stigma 2-lobed. Fruit is a small, one-celled capsule with a transparent yellowish pericarp, superior, bicarpellary. It dehisces from above, septicidally into two valves. Seeds are numerous, minute, irregularly ovoid, many-sided and angular. The plants are in bloom between the period of September and October. The flowers are greenish in color with a purple tinge and hermaphrodite in nature.

Odour is absent. All parts of the plants are extremely bitter. The true chirata can be distinguished from other substitutes and adulterants by its intense bitterness, brownish–purple stem (dark colour), continuous yellowish pith and petals with double nectarine.

Earlier morphological evidences represented cross-pollination in the species and bees (Apoidae, Hymenoptera) were considered to be the pollinators of *S. chirayita* (Khoshoo and Tandon, 1963). However later findings contradict the earlier reports. The findings of cytological studies and bagging experiments confirmed that the probable mode of pollination is self-pollination and chances of cross-pollination vary only between 16-20% (Chakraborty *et al.*, 2009).

Recent findings, however, suggests that the mode of pollination appears to be mostly cross pollination, but self pollination is also possible. This is of great advantage to this species because of its endangered status. Out crossing would ensure creation of new gene combinations which will help its stock in overcoming the vagaries of fast climate change. Production of inbred seed appears to be a “feel safe” strategy to produce seeds when pollinators are scarce (Shah *et al*., 2011).

Fig. 2.1 A: A. flowering twig. B. Tetramerous flower of Chirata

2.1.8.2. Microscopic evaluation

2.1.8.2.1. Root

Transverse section of root shows, 2-4 layers of cork, thick-walled parenchymatous 4-12 layers of secondary cortex cells with mucilage, minute acicular crystals (also present in phloem region), and resin (as dark brown mass); secondary phloem composed of thin-walled strands of sieve tubes, companion cells and phloem parenchyma; lignified and thick-walled scalariform, simple and bordered pitted secondary xylem vessels, tracheids, parenchyma and xylem fibers. Minute acicular crystals present in abundance in secondary cortex and phloem region.

2.1.8.2.2. Stem

Transverse section of stem shows single-layered epidermis, showing anticlinal or periclinal walls; parenchymatous cortical cells with mucilage, minute acicular crystals, resin (as dark brown mass), and oil droplets; endodermis single, thin-walled pericycle; rounded and isodiametric pith cells with prominent intercellular spaces; xylem composed mostly of tracheids, fibers and a few vessels, mostly single or rarely in groups of two, amphiphloic siphonostele, medullary rays absent; prominent intercellular spaces present in pith cells.

2.1.8.2.3. Leaf

Transverse section of leaf shows single epidermal layer covered with a thick, striated cuticle, more strongly developed on the upper surface than the lower; anisocytic stomata; single layered palisade tissue, spongy mesophyll cells represented by 4-7 layers of somewhat loosely arranged, elongated cells. Mucilage and minute acicular crystals present in abundance in mesophyll cells.

2.1.9. Description of drug

Chirata of the market consists principally of short sections of the stem and branches. The stems contain yellowish pith. The drug is officially described as: “stem about one meter long, smooth, brown, or extremely yellowish, purplish–brown, slightly winged and much branched above, rounded below and containing large yellowish,

continuous, easily separable pith. Root simple 7mm in thickness near the crown, oblique. Branches slender, elongated, decussate. Leaves sessile, ovate-lanceolate, opposite, glabrous, entire, usually with 3-7 lateral veins, about 6cm in length. Flowers small, numerous, panicled, with a 4-lobed calyx and corolla, capsule ovoid-acute, one-celled, many-seeded. Fruits superior, bicarpellary, unilocular. No odour; taste extremely bitter; wood yellowish, thin. Chirata yields not more than 6 percent of ash."

2.1.9.1. Powder

Greyish brown. Characteristic elements include parenchyma of medulla, slightly lignified with simple pores; sclerenchyma with fibres, long, narrow, and thick-walled; tracheids, numerous; ducts with spiral or scalariform markings; yellowish-brown pollen and stomata present.

2.1.10. Domestication and propagation

Despite a descent hold in the herbal industry, chirata is still collected from the wild; it is sparsely cultivated and negligible efforts have gone into developing proper agro-techniques of the plant. This plant can be grown easily on fields or even on the sloppy areas and is distributed from far East to far West. Chirata grows in association with other native vegetation in open and dry areas of degraded broad leaf forests, fallow dry land and grazing areas. It is also found in open ground and recently slash-and-burnt forests.

Chirata thrives as well as flourishes in woodland gardens having a sunny edge, partial shade, in shade as well as in marshy lands. This plant has a preference for sandy (light), loamy (medium) as well as clay (heavy) soil conditions. In addition, it thrives and flourishes well in acidic, neutral as well as basic or alkaline soils. It can grow well in semi-shade or somewhat woodland conditions and needs humid or damp soil, preferably in woodlands along the streams or in marshlands. The plant actually develops best in areas where the summers are cool. Hence, it is no surprise that chirata can thrive and flourish both in conditions where there is full sunlight as well as partial shade. The chirata plants are able to withstand temperatures as low as -15° C and still continue to grow well.

S. chirayita is propagated by its seeds. Seed setting commences around October to November when the seed matures and drops and in the months of June to July next year

the seed starts germinating. Sowing is generally done during spring when the temperature is not above 10°C and in a situation when the soil contains plenty of humus. When the seedlings have grown adequately to be handled, they are taken out individually and planted into separate pots or containers. The young plants are re-planted outdoors during the early part of summer (Kumar *et al*., 2010).

Seed germination is a major obstacle in domesticating this species. Only a few scattered reports in the literature suggest germination studies and nursery practices of *S. chirayita.* Seeds of chirata need some sort of physiological pre-conditioning before germination which is provided by very low temperature. Ninety-one per cent seed germination was reported after 3^0C chilling treatment for fifteen days, whereas another study reported a maximum of 81% germination (Raina *et al*., 1994; Basnet, 2001). An observation at the post-germination growth stage revealed that *S. chirayita* is a slow-growing species (Basnet, 2001). In another study, it was found that physiological dormancy can be broken by pre-sowing chemical treatments.

Germination of seeds of chirata in natural conditions is erratic and the dormancy period is long. Pre-sowing chemical treatments are used to enhance seed germination of wild sources of several Himalayan medicinal plants (Nadeem *et al.,* 2000; Pandey *et al*., 2000, Joshi and Dhar, 2003; Manjkhola *et al.,* 2003; Butola and Badola, 2004a, b, 2006a, 2007; Shivkumar *et al*., 2006) and in plants of other regions (Plummer and Bell, 1995; Yamaguchi and Kamaya, 2001; Ghimire *et al.,* 2006; Kulkarni *et al.,* 2007; Vandelook *et al.*, 2007; Kaur *et al.,* 2009).

The above studies were mostly confined to test seeds from wild. Seeds of only a few endangered Himalayan medicinal herbs have been evaluated, following establishment of *ex situ* set-ups, for their germination potential assessment, especially using various chemical stimulants and growing conditions (Butola and Badola, 2006a, 2007). Low germination percentage and viability of the seeds, long gestation periods and delicate field-handling are some of the factors which discourage commercial cultivation of the plant (Badola and Pal, 2002).

Ex-situ produced chirata seeds were subjected to 11 pre-sowing chemical treatments, among which Gibberellic Acid (50 to 350 µM) most effectively stimulated seed germination (96.7%, maximum; $p<0.001$) and reduced mean germination time ($p<0.05$). Study confirms *ex situ* produced seeds attained physiological dormancy, which

was broken by pre-sowing treatments, as a tool to *ex situ* species conservation (Pradhan and Badola, 2010).

The effect of GA_3, IAA and KNO_3 on the improvement in seed germination of *Swertia angustifolia,* which is often used as a substitute of *Swertia chirayita*, was studied by Bhatt *et al.*, 2005. The results showed that GA_3 was found to be the best with respect to germination (96.0%) and reducing mean germination time (7.6 days) followed by KNO_3 (81.3%; 8.4 days) and IAA (66.0%; 16.6 days). Germination of the species under controlled conditions is found to be low (< 32.0%).

2.1.10.1. Micropropagation

The novel technique of *in vitro* conservation and micropropagation can help in conservation and production of a large number of disease-free, true-to-type plants. Wawrosch *et al.* (1999) reported shoot regeneration from root explants. Ahuja *et al.* (2003) have optimized media condition for faster propagation of *S. chirayita.* Attempts have been made to standardize root cultures for production of active metabolites under *in vitro* conditions (Keil, 2000). Root culture studies have been taken up in related species of *Swertia* (Ishimaru *et al.* 1990; Kitamaru *et al.* 1988).

Axillary multiplication from 4-week old seedling-derived nodal explants resulted in 4.5-fold multiplication every 4 week and a success rate of 94% was obtained by *in vitro* hardening in the growth-room and by *ex vitro* hardening in greenhouse conditions (Joshi and Dhawan, 2007).

Balaraju *et al.* (2009) developed an efficient *in vitro* plant regeneration protocol for using shoot tip explants derived from *in vitro* grown seedlings. Various hormones such as 6-Benzyl-amino purine, Kinetin, Thidiazuron, Naphthalene Acetic Acid were used. Pant *et al.* (2010) has developed a procedure for *in vitro* propagation through axillary bud culture of chirata. Jha *et al.* (2011) reported somatic embryogenesis from leaf explants of *in vitro* grown shoots. Wang *et al.* (2009) reported shoot regeneration from the leaves with the effects of phytohormones and medium. *In vitro* regeneration of plants from root cultures was obtained. Regenerated shoots were further multiplied on full strength MS medium supplemented with different concentrations of plant growth regulators (Pant *et al.,* 2010).

Chaudhuri *et al.,* 2008 reported direct induction of more than seven shoot buds per explant using leaves taken from in vitro shoot cultures on MS medium supplemented with 2.22 μM N-6-benzyladenine, 11.6 μM kinetin, and 0.5 μM α-naphthalene acetic acid. Plants raised through direct organogenesis were evaluated for their clonal fidelity by chromosomal analysis and DNA fingerprinting. Balaraju *et al.*, 2010 showed maximum germination and percentage of survival with the medium containing 2,4-D in combination with BAP after embryogenic tissue initiation with 2,4-D.

However, there exists a need to translate these *in vitro* studies to the field for practical applications. The conventional methodology for tissue culture is not so efficient for producing high biomass volume with all time risk of contamination and limitation in scaling up. So to overcome the problem, the technical advancements are needed to be improvised for getting some possible breakthrough in commercial protocols using temporary immersion system which will be most promising for industrial plant propagation because of its prominent technology in reducing the labour and providing low production cost for obtaining large amount of biomass of *Swertia chirayita*. Commercial propagation of *Swertia chirayita* using this system showed 3-4 fold increase in rate of multiplication and biomass increase (Kumar *et al*., 2013).

2.1.11. Harvesting

The plant is harvested for the drug industry when it sets into flowering in July to September (Anon, 1982; Bentley and Trimen, 1880). The plant is gathered during the late stages of flowering, commonly tied up in flattish bundles (Fig. 2.2), about 3 feet long and 1.5 to 2 lbs in weight (Bentley and Trimen, 1880). Farmers collect the entire chirata plant during the months of December-January. The best quality can be harvested just after flowering is over and before seed production. Since it is believed that the roots contain the highest proportion of medicinal value, the plants are uprooted. However, harvesting the plant before seed dispersal has negative impacts on the natural regeneration and this practice is strongly discouraged.

2.1.11.1. Post harvesting treatment

The fresh uprooted plants are tied into small bundles for transportation, and dried in direct sunlight by spreading them on bamboo mats or jute bags on the ground. This

method is labour intensive and time consuming. Drying in the sun takes about one week. Shade drying is less frequently done. However, when there is not enough sunshine farmers will stack the plants at the attic of their houses. This method often creates favorable conditions for development of fungus and bacteria, and results in a low-quality product. The drying process is the most important factor influencing the quality and price of chirata. The dried products are kept in the attic until they are transported to the market. The collection of chirata for commercial purposes began when farmers realised that it has economic value. After the harvesting season, when farmers have gathered and dried all their products, the bundles are baled into appropriate size packages and transported to the local market. Farmers can lose about 30% of their products between the time of harvesting and selling because of improper management of drying, packaging and storage.

Quality criteria for chirata are as follows:

- reddish brown in color (not black);
- very bitter in taste (to differentiate mixture of species);
- well air-dried (100% on air dry basis); and,
- not infected by fungi (mycelium growth).

Fig. 2.2 Harvested bundle of *Swertia chirayita*

2.1.12. Medicinal uses

Although almost all the varieties of chirata are medicinal, nonetheless *Swertia chirayita* (locally known as bitter or original chirata) contains more bitterness as compared to other varieties, and is hence more valuable or useful. The entire plant is used in traditional medicine; however the root is mentioned to be the most powerful part (Kirtikar and Basu, 1984). *S. chirayita* is used in British and American pharmacopoeias as tinctures and infusions (Joshi and Dhawan, 2005). The widespread use of this plant in different alternative systems of Indian medicines like Ayurveda, Unani, and Siddha reflects its pharmacological importance. The medicinal properties of chirata are reported in the Indian Pharmaceutical Codex, the British and the American Pharmacopoeias.

2.1.12.1. Action and Uses

The properties of chirata are those of the pure bitters, and probably do not differ from those of the other members of the Family Gentianaceae. In overdoses, it nauseates and oppresses the stomach. Some have supposed that, in addition to its toxic properties, it exerts a peculiar influence over the liver, promoting the secretion of bile and correcting it when deranged, and restoring healthy evacuations in cases of habitual costiveness. It has been used in dyspepsia, and in the debility of convalescence, and generally in cases in which corroborant measures are indicated. It may be given in powder, infusion, tincture, or fluid-extract. Powdered drug is used as an ingredient of Sudarshana Churna, a tonic and a febrifuge in the Ayurvedic System. Dosage: 0.6-2g, occasionally 10-30g (Wealth of India, 1952; The Indian Pharmaceutical Codex, 1959; Wallis, 1967). The whole plant-stem, leaves, flowers and roots-are used as medicines in Indian Ayurvedic System.

- Traditionally, *Swertia* species are used as laxative, cathartic, analeptic, febrifuge, stomachic, anti-malarial, expectorant, bitter digestive tonic (Kirtikar and Basu, 1933; Chopra, 1933). Dosage: 1-3g of the drug in powdered form, 2-4ml concentrated compound chirata infusion and 15-30ml of compound chirata infusion are prescribed for use as bitter tonic.
- The dried whole plant biomass has long been used for various ailments like diarrhoea, cough, all types of fevers, anaemia, gastropathy, dyspepsia, burning of the body and pain in the joints and skin diseases (Rastogi and Mehrotra, 1994; Sundriyal and Sharma, 1994) etc. It was also used as a remedy for scanty urine,

constipation, urinary discharges, ulcers, leucorrhoea, gastrointestinal diseases, hiccup, kidney diseases, urinogenital tract disorders and epilepsy and for certain types of mental disorders. The water extract of the plant is used as a liver stimulant and in combination in bronchial asthma and debility. It is even used for relaxation to pregnant uterus (Kirtikar and Basu, 1984), as purifier of breast milk and as carminative (Garg, 1965 and Sharma, 1986).

- Chirata is even used for treating cancer. It is used as a breath refresher and to reduce vomiting during pregnancy. Traditional Bhutanese medicine also uses chirata for blood purification and to cure common cold, gout disease and diabetes. In rural areas, the plant is chopped in small pieces and soaked in water for one night before it is boiled and consumed in the form of tea. In general, a small tea cup twice daily is prescribed. Chirata is excellent for de-worming children and for this purpose it should be taken every morning for a number of days, then discontinue for a time and resume taking the chirata tea again until the worms are gone. The stem of chirata used in combination with other drugs is also prescribed in the treatment of scorpion stings but it is not an antidote to scorpion venom.

2.1.12.2. Action and Uses in Ayurveda and Siddha

According to Ayurvedic Pharmacology (20), chirata is described as bitter in taste (*rasa*). The thermal action (*virya*) of chirata is defined as cooling (*shita*). Chirata is light (*laghu*), *i.e*. easily digestible, and dry (*ruksha*). These characteristics drain heat from the blood and liver. Concoction of chirata with cardamom, turmeric and kutki is given for gastrointestinal infections, and along with ginger it is considered good for fever. When given along with neem, manjishta and gotu kola, it serves as a cure for various skin problems. It is used in combination with other drugs in cases of scorpion bite (Nandkarni, 1976). Ayurvedic practitioners prescribe this infusion in doses of two ounces twice a day before meals as a tonic to check hiccup and vomiting.

2.1.12.3. Action and Uses in Unani

According to Unani system of medicine, chirata is used as a tonic to heart, liver and eyes. It is used to cure cough, scanty urine, sciatica, skin diseases, melancholia and dropsy. It is drying, liquefying and acts as resolvent and astringent. An infusion of the

herb made in hot water with aromatics like cloves, cinnamon etc is given in doses of half to one fluid ounce. Dosage: An infusion of the herb is generally employed. It is also given as tincture. Its decoction is not recommended. The root is taken in doses of 5-30 grains with honey. This herb is used as a part of many compound remedies.

2.1.13. Pharmacology

The ethanolic extract of *S. chirayita* exhibits hypoglycaemic activity. The hexane fraction containing swerchirin, the main hypoglycaemic principle, induced a significant fall in blood sugar in albino rats. The compound may have clinical application in control of diabetes. It also possesses anti-microbial activity against gram-negative and gram-positive bacteria. The methanol extract of *S. chirayita* was found to have antihepatotoxic activity against carbon tetrachloride induced liver toxicity in experimental rats (Karan *et al.*, 1999). The pet-ether fraction of *S. chirayita* showed sustained hypoglycaemic activity on *Swiss albino* mice. This observation confirms the use of chirata in ethnomedical application for diabetes management (Alam *et al.*, 2011).

The extract of the plant exhibits a significant anti-inflammatory activity. The plant extract shows antileishmanial activity against *Leishmania donovani* in golden hamsters. Laboratory tests with animals having excessive baseline blood sugar levels have demonstrated diminished blood sugar levels following healing with chirata. On the contrary, animals do not demonstrate such decrease in the blood sugar levels provided they already have low levels to begin with. This difference in results in treatment with chirata provides an indication that the herb may perhaps be beneficial in regulating blood sugar levels without the perils of developing hypoglycaemia owing to any excessive dosage of the herbal medication. Additional animal studies with chiretta have discovered that this herb is more effectual in regulating blood sugar levels compared to the regular anti-diabetic drug Orinase (Tolbutamide).

The aqueous extract of *Sudarshanchurna,* an Ayurvedic herbal formulation (containing *Swertia chirayita* 50%), claimed traditionally for antipyretic and antimalarial properties, produced significant reduction in elevated body temperature on Brewer's yeast induced pyrexia in albino rats and typhoid-paratyphoid A, B vaccine induced hyperexia in rabbits and was comparable to that of paracetamol, a standard antipyretic agent. The result

showed antipyretic potential of the formulation. Similar result was obtained with the aqueous extract of chirata root (Bhargava *et al.,* 2008, 2009).

The ethanol extract of leaf, stem, and their different fractions i.e. pet-ether, dichloromethane, and methanol fraction of chirata showed analgesic activity on acetic acid induced writhing in *Swiss albino* mice (Alam *et al*., 2010). The ethanolic extract of chirata leaves reversed anaemia, induced by phenyl hydrazine in rats. The action is similar to those induced by parasite such as *Plasmodium falciparum*. The vitamin and mineral constituents of the leaf appear most likely as the active ingredients responsible for the haematinic effect of chirata leaves. This result supports at least partially the traditional use of chirata in the treatment of anaemia (Turaskar *et al*., 2013).

The ethanolic extract was shown to possess antidiabetic activity on Streptozotocin-Nicotinamide (STZ)-induced diabetic albino mice (Arya Renu *et al.,* 2011). Amarogentin, a secoiridoid glycoside isolated from the plant, was evaluated in free and two different vesicular forms, liposomes and niosomes, in a hamster model of experimental leishmaniasis. It showed antileishmanial property. The therapeutic efficacy of amarogentin in both the forms was found to be more active than the free amarogentin (Medda *et al.,* 1999). Chirata showed antiviral properties against Herpes simplex virus type-1 (Verma *et al.,* 2008).

Anti-inflammatory action of aqueous suspension of total xanthones of *Swertia chirayita* was suggested by Chowdhury *et al.,* (1995). Further studies on the antiinflamatory effects of orally given aqueous suspension of total xanthones as compared to the standard antiinflamatory drugs phenylbutazole and betamethasone, revealed that chemically induced hind paw oedema in rats could be suppressed significantly (Mandal, 1997). The whole plant methanol and aqueous extracts of *Swertia chirayita* possessed maximum anti-inflammatory activity in a dose dependent manner in carrageenan-induced animal models (Mathur *et al.,* 2011). A study by Neetu *et al.* (2013) indicated that the methanolic extracts of chirata showed good antioxidant activity, and can therefore be proposed as new potential sources of natural additives for the pharmaceutical industries.

An earlier study by the same group (Mandal *et al.,* 1992) had reported that benzene extract of the plant containing a mixture of xanthones was effective in reducing acute, sub-acute and chronic types of inflammation both on immunological and non-immunological models. Antihelmintic activity of aqueous and methanolic extracts of *Swertia chirayita*

reported by many (Iqbal *et al.,* 2006) may be accounted for by the presence of amarogentin as well as other secoiridoid glycosides like amaroswerin and sweroside.

Extracts and many chemical constituents of the plant have exhibited antipyretic, antihelmintic, antiperiodic, antihepatotoxic, antiinflamatory, antiulcerogenic, anticholinergic, CNS depressant, antimalarial and hypoglycaemic activities in animal models. Herbal medicines such as Ayush-64, Diabecon, Mensturyl syrup and Melicon V ointment (Edwin *et al.,* 1988; Mitra *et al.,* 1996 and Valecha *et al*., 2000) contain chirata extract in different amounts for its antipyretic, hypoglycaemic, anti-fungal and anti-bacterial properties. In recent clinical investigations, the drug was found useful in different types of jaundice and especially useful in hepatitis. The drug is also used in chlorotic action and is useful in infective and amoebic type of inflammation in liver.

2.1.14. Economic importance

Swertia chirayita has an established domestic (Indian) and international market, which is increasing at a rate of 10% annually. The medicinal plant sector in India is unorganized and it is difficult to get a regular update of statistics *vis-à-vis* the demand and supply, collection and economics of chirata. It may be because of the knowledge of importance of chirata that its cultivation has been started. The plant has a huge demand in the medicinal market and is an important factor for the economy of Eastern Himalayas (Darjeeling and Sikkim). Easy growing plant on the field, or even on open grasslands, chirata should be conserved; and if this knowledge is made available at the local level, then there are great possibilities of developing chirata into an economical plant or a source of income. At present, chirata is priced at Rs. 400-500 per kg at the local market.

2.1.15. Secondary metabolites / Major chemical constituents

Reviews detailing the chemical constituents of the *Swertia* genus have been reported (Wang and Yang, 1992; Rahman and Arfan, 1997; Pant *et al*., 2000). *S. chirayita* belongs to family Gentianaceae, which records the occurrence of taxonomically important groups of secondary metabolites, namely iridoids, xanthones, mangiferin and C-glucoflavones. The bitterness, antihelmintic, hypoglycaemic and antipyretic properties are attributed to amarogentin (phenol carbonic acid ester of sweroside, a substance related to gentiopicrin), which constitute about 0.04%, amaroswerin about 0.03%, swerchirin, swertiamarin, gentianine and gentiocrucine (Joshi and Dhawan, 2005).

Among the different seco-iridoids present in *S. chirayita*, swertiamarin is one of the most predominant and have well-established, important therapeutic applications like CNS depressant and anticholinergic effects and was found in amounts of about 30% on fractionation and purification of the extract. A simple, rapid, and accurate High-Performance Thin-layer chromatographic (HPTLC) method has been developed for the determination of swertiamarin in aerial parts of *Swertia chirayita* (Anjum *et al.,* 2014).

It also contains yellow crystalline substance used in dyeing. Important phytochemicals like amarogentin and swerchirin have been investigated for drug reinforcement (Brahamchari *et al.,* 2004). Amarogentin is, in fact, the bitterest compound isolated from natural products till date (Karan, Vashist and Handa, 1996). The drug further contains apocyanin, a number of xanthone derivatives, *viz.*, methyl bellidifolin, triterpenoids, *viz.*, masilinic acid.

Seven xanthones together with ursolic acid could be isolated from this natural source. Number of xanthones, 1,5,8-trihydroxy-3-methoxy xanthone, 1-hydroxy-3,5,8 trimethoxy xanthone, 1-hydroxy-3,7,8-trimethoxy xanthone, 1,8-dihydroxy-3,5-dimethoxy-xanthone, 1,8-dihydroxy-3,7-dimethoxy xanthone, 1,3,5,8-tetrahydroxy xanthone, C-2-β-D-glycoside (mangiferin), 1,3,8-trihydroxy-5-methoxy xanthone, 1,3,7,8-tetrahydroxy xanthone, a novel dimeric xanthone-chiratanin, a number of triterpenes including swertanone and the alkaloids gentianine, gentiocrucine and enicoflavine were isolated. The isolation and characterization of a new xanthone, in addition to the two 1, 8-dihydroxy-3, 5-dimethoxy xanthone (swerchirin) and 1, 8-dihydroxy-3, 7-dimethoxy xanthone (7-O-methyl swertianin), were reported earlier from this plant. The presence of xanthone 6 (1, 5, 6-trihydroxy-3-methoxy xanthone) was observed for the first time in this genus.

A new interesting observation is the presence of dimeric xanthone designated chiratanin in this species, and this is the first report of the occurrence of dimeric xanthone in higher plants. Gentiopicroside considered as characteristic water soluble bitter glucoside of Gentianaceae is found in many species of *Swertia*. The structure of gentiopicroside was only recently proved by structural relationship with gentianine, an alkaloid present in *Gentiana* and also obtained by reaction with ammonia from gentiopicroside. Swertiamarine, an important chemical found in *Swertia* species is chemically 5-hydroxygentiopicroside.

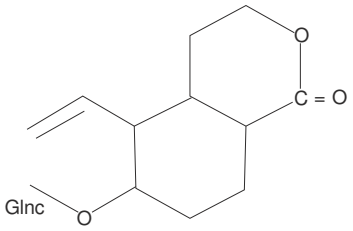

Fig. 2.3 (a): Structure of Gentiopicroside

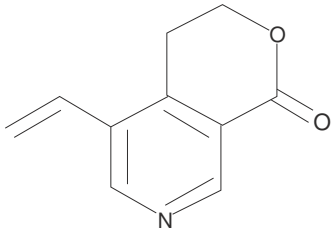

Fig. 2.3 (b): Structure of Gentianine

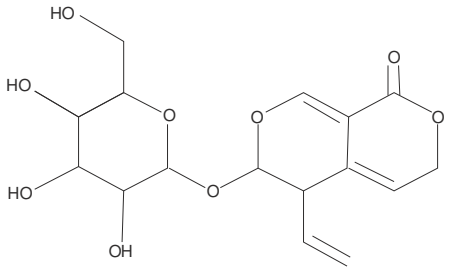

Fig. 2.3 (c): Structure of Gentiopicrin

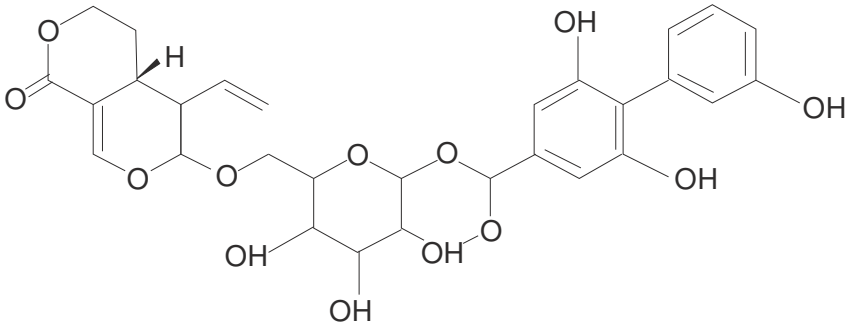

Fig. 2.3 (d): Structure of Amarogentin

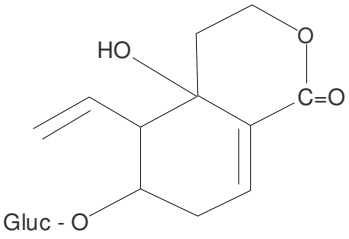

Fig. 2.3 (e): Structure of Swertiamarine

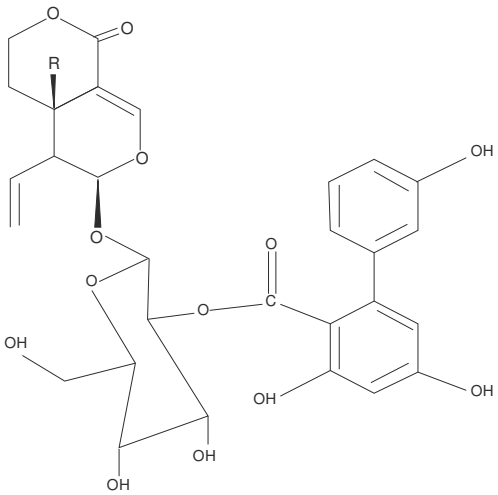

Fig.2.3 (f):R=H (Amarogentin)
R=OH (Amaroswerin)

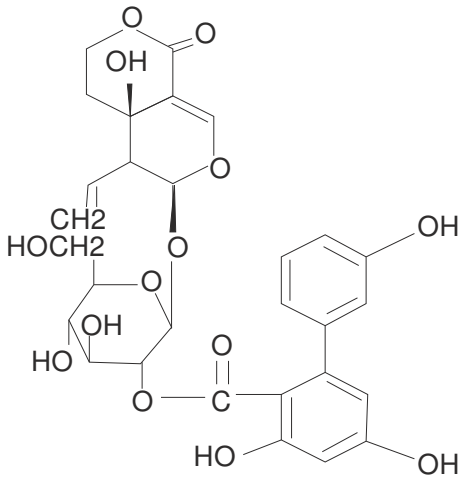

Fig. 2.3 (g): Structure of Amaroswerin

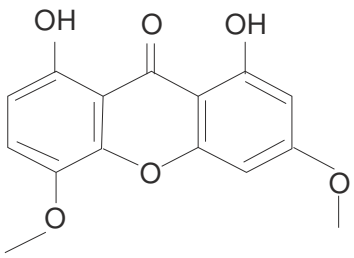

Fig. 2.3 (h): Structure of Swerchirin

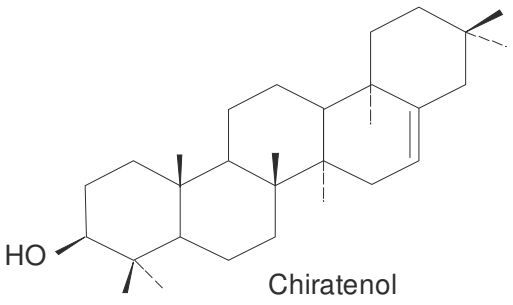

Fig. 2.3 (i): Structure of Chiratenol

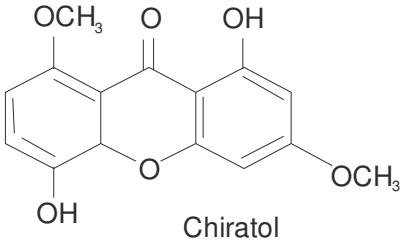

Fig. 2.3 (j): Structure of Chiratol

Hohn (vide Anonymous, 1952) reported that chirata contains two bitter substances, a bitter glucosidal principle chiratin ($C_{26}H_{48}O_{15}$) and a bitter ophelic acid ($C_{13}H_{20}O_{10}$)**.** Chiratin is a yellow, hygroscopic powder, but feebly crystallizable, very bitter, sparingly soluble in cold water, more so in hot water, and readily dissolved by alcohol and ether. It is neutral to test paper, and yields a copious precipitate with tannic acid. By the action of acids, chiratin is separated into ophelic acid, and yellowish-brown, amorphous substances, bitter, scarcely soluble in water, readily soluble in alcohol, and not reducing copper solutions (as the ophelic acid does). Hohn gave it the formula $C_{13}H_{24}O_{3,}$ and named it chiratogenin. It is unaffected by tannin. Ophelic acid is a hygroscopic, non-crystalline, yellow, viscid body, having an odour faintly suggestive of gentian and an acidulous, bitter taste which is persistent. The acid is syrupy, deliquescent, yellowish-brown, at first slightly sour, afterwards intensely bitter. It is soluble in water, with some turbidness, probably owing to resin mixed with it, and completely soluble in alcohol, or a mixture of this with ether. It decomposes certain salts, and forms amorphous compounds with acids. Basic lead acetate precipitates it yellow.

It also contains oleanolic acid, which has anti-ulcer and analgesic activities and inhibits 5-α-reductase which is related to oleopecin. It also has angiotelectase activity (Khetwal and Verma, 1984). Mazumder and Guha (1933) isolated a phenolic compound ($C_{13}H_{14}O_5$), a neutral pale yellow crystalline compound ($C_6H_8O_3C$), m. p. 196-198ºC, oleic, palmitic and stearic acids, a phytosterol, a monohydroxy acid, and a large amount of resinous matter in addition to the compounds isolated by Hohn. Freindhelm (vide Prasad *et al.,* 1960) noted the presence of gentiopicrin and amarogentin ($C_{32}H_{38}O_{16}$), m.p. 178-180ºC. Dalal and Shah (vide Prasad *et al*., 1960) isolated swerchirin, a new xanthone from this plant ($C_{25}H_{12}O_6$; m.p. 185ºC; Wealth of India, 1952; Wallis, 1967).

The ash of chirata yields carbonate and phosphates of calcium, potassium and magnesium. Tannin is almost entirely absent. A crystalline, yellow, waxy body in small amount, as well as the ordinary plant constituents, abound. According to Lassaigne and Boissel, the stems contain resin, a yellow bitter substance, brown colouring matter, gum, and various salts. Because of the water solubility property of these chemical constituents, a light brown to coffee colour is obtained when any part of chirata is kept immersed in water or is boiled with water.

Quantification of major phytochemicals, mangiferin, amarogentin and swertiamarin, showed that the highest quantity of all the three phytochemicals was found

in inflorescence and leaf mixture of all the collected plant samples. There was no significant difference in the amounts of these three phytochemicals between extracts from wild and cultivated plants, which substantiates the validity of cultivated *Swertia chirayita* for medicinal purposes and trade (Phoboo *et al.,* 2010).

2.1.16. Conservation

The rural people collect chirata directly from the wild populations of the forest, meadow, scrub or shady habitats which are already dwindling due to over-exploitation and unsustainable land-use, thus accelerating their genetic erosion. Out of all the species, *Swertia chirayita*, the one which is commercially more important; is in the danger of getting lost or depleted from its natural habitat. Considerable importance of this crop coupled with lack of organized cultivation has created a lot of pressure on its natural resources which has led to considerable depletion of its stock. Unsustainable collection of the species has been done due to their usefulness to cure various ailments, their increasing price, and increasing demand as raw materials for preparation of Ayurvedic and Allopathic medicines.

Extensive collection and unscientific harvesting practices of these plants from the natural habitat leads to an increasing danger of extinction (Bhattarai and Shrestha, 1996; Edwards, 1993; Joshi, 2008). The present rate of exploitation has rendered some species to the status of threatened and endangered species. Serious threats to the population of *Swertia* were noticed due to habitat destruction and land use change. Even without tree-removal, extensive grazing of domestic animals in the forests can be damaging to the species. The existing populations of chirata are reported to be diminishing.

Keeping in view the importance and conservation value of *S. chirayita*, some conservation measures need to be taken, i.e. notify the natural populations for the protection of its habitats, detailed study on phenology with understanding of whole life cycle, sustainable collection of germplasm from natural populations for developing elite generations in future, rehabitation of species before taking any developmental activities, establishment of nurseries in nearby areas of natural habitats, development of large-scale seedlings and their plantation, restrictions in field survey and collection in sensitive areas having natural populations and creation of awareness among the local healers to control over-exploitation in natural habitats to maintain its posterity for future (Purohit *et al.,* 2013).

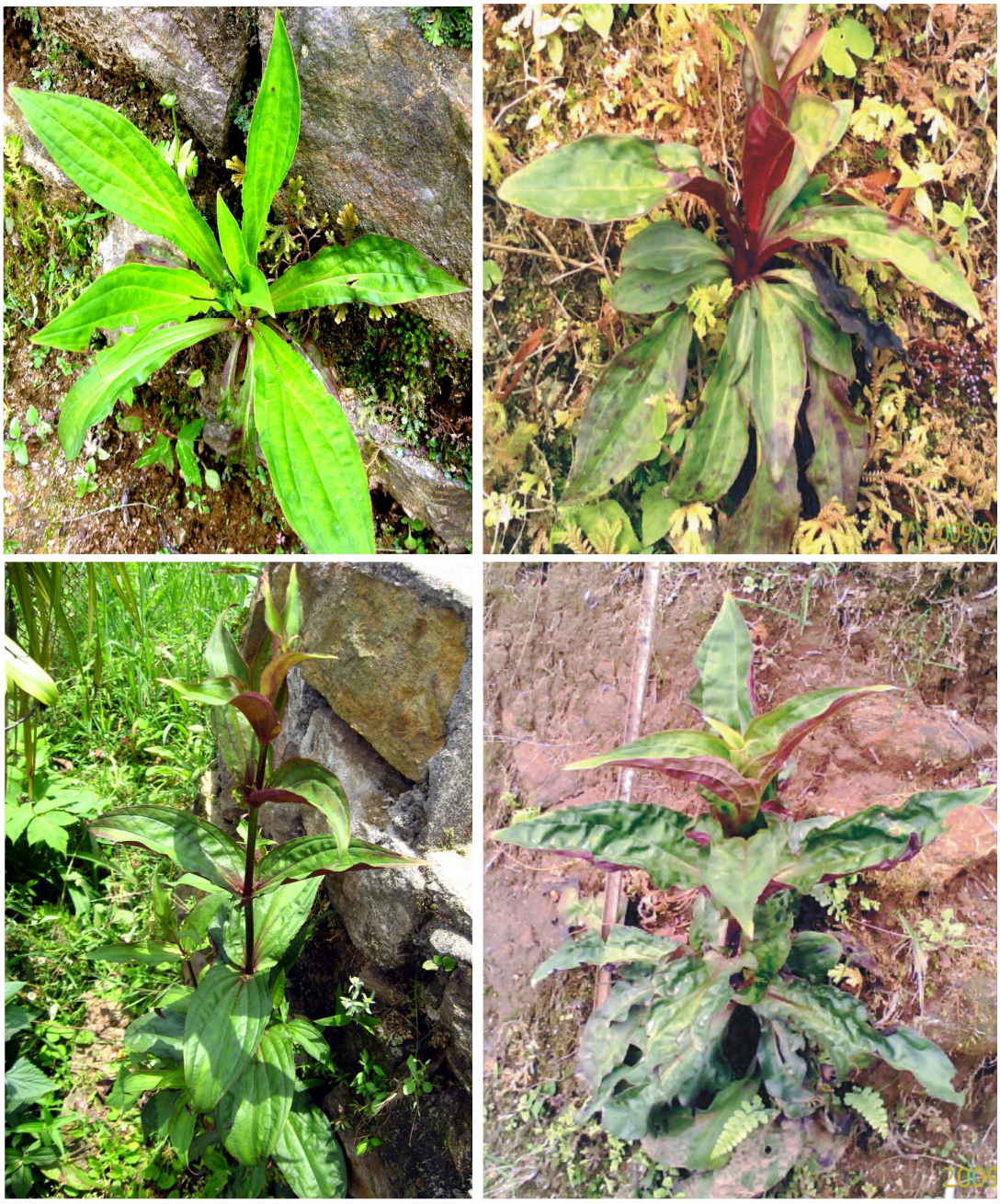

Fig. 2.4: Naturally growing *Swertia chirayita*

2.1.17. Adulterants

The plant available in the market, many a times, is adulterated and substituted by close relatives of chirata. Adulteration of chirata with other low quality species of *Swertia* and other related species are very common in the trade of chirata. However, the large continuous stem pith, dark green colour of stem and intensely bitter taste of leaves are sufficient to distinguish *S. chirayita* from other species of this genus, which are used as adulterants (Anonymous, 1976). *Swertia angustifolia* Buch.-Ham. ex D. Don, is the most common adulterant of *S. chirayita.* The bitter tonic property of the adulterant is much inferior as compared to *S. chirayita*. The two plants resemble to a great extent with reference to morphological and anatomical features. *S. chirayita* has a stomatal index of 19.90 and average stomatal number of 118 per sq. m., whereas the respective values for *S. angustifolia* are 11.99 and 86 per sq. m. *Andrographis paniculata* which is chemically also different from *S. chirayita,* in having andrographolide as the major constituent, often gets substituted for or confused with *S. chirayita*.

The trade and economics of chirata is also affected by adulterants of the herb. *Andrographis paniculata* (green chirayita), *Exacum tetragonum* Roxb., *E. bicolor* Roxb., *E. pedunculatum* Linn., *Slevolgia orientalis* Griesb., *Swertia alata* Royle., *S. angustifolia* Buch.-Ham., *S. bimaculata* Hook. f. and Thoms., *S. ciliata* G. Don, *S. densifolia* Griesb., *S. elegans* Wight., *S. lawii* Burkill., *S. minor* Griesb., *S. paniculata* Wall., *S. multiflora* Dalzell are adulterants found along with true chiretta. *S. minor* Griesb. is used as a substitute for chirata in treatment of malaria and other fevers. However, substitutes are inferior to *S. chirayita* in terms of bitterness (Anon, 1982).

Several species of *Ophelia,* such as *O. angustifolia* Don (less bitter than chirata), *O. elegans* Wight, *O. densifolia* Grisebach, *O. multiflora* Dalz, *O. pulchella* Don, and related plants go by the name of chirata in the Indian bazaars. These are designated by the natives as hill (*paharee*) chirata, sweet (meetha) chirata, purple (ooda) chirata, and southern (dukhunee) chirata. Chota chiretta or small chiretta is the product of *Slevogtia orientalis* Griesbach. These all possess, more or less, the bitter virtues of chirata. Paharee or hill chirata is distinguished by its inferior bitterness, and its rectangular, winged stems, whose section presents a thick woody ring and a centre nearly or entirely hollow, with only traces of pith.

A false chirata, which also found its way into the London markets, and resembles the official variety in having well developed pith, but which is completely lacking in bitterness, is affirmed to be the product of *Ophelia alata*. Under the name of Indian chirata, the dried plant of *Andrographis paniculata* Nees has appeared in the London market. It resembles much more closely recently dried broom tops than the true chirata. It is likely more than 2 feet long. The branching stems are from 1/8 to1/4 inch in thickness near the base, woody, quadrangular, furrowed, smooth, slightly knotted at the point from which the branches spring, the longitudinal furrows are continued through the roots, which have numerous fine radicles; the leaves are opposite decussate, branches erect or forming an acute angle with the stem, terminal shoots extremely slender.

2.2. PLANT GROWTH SUBSTANCES

Plant growth regulators are chemical substances and when applied in small amounts, they bring rapid changes in the phenotypes of the plant and also influence the plant growth, right from seed germination to senescence either by enhancing or by stimulating the natural growth regulatory system. Plant growth substances are known to enhance the source-sink relationship and stimulate the translocation of photo-assimilates thereby helping in effective flower formation, fruit and seed development and ultimately enhance the productivity of crops. Growth regulators can improve the physiological efficiency including photosynthetic ability and can enhance effective partitioning of accumulates from source and sink in the field crops. Foliar application of growth regulators and chemicals at the flowering stage may improve the physiological efficiency and may play a significant role in raising the productivity of the crop (Amanullah *et al*., 2010).

Plant hormones exert far reaching effects on plant growth, the precise action depending on the concentrations of the substances present and the sensitivity of the organ concerned. Growth regulators play an important role in both morphology and physiology of the plants. The effect of growth regulator varies with plant species, variety, their growth stage, concentration of chemicals, application method and frequency of application (Hilli *et al*., 2010).

Plant growth regulators influence the plant growth when applied in very minute quantity. There are many reports which indicate that application of growth regulators

enhanced plant growth and crop yield (Hernandez, 1997; Ashraf *et al*., 1987, 1989). Lee *et al*., (1999) reported that GA_3 increased stem length and number of flowers per plant. Kabar (1990) found that GA_3 accelerated bud development and stem elongation but the best results can be achieved if GA_3 is applied in combination with kinetin. IAA exerts influence on plant growth by enlarging leaves and increasing photosynthetic activities in plants. It also activates the translocation of carbohydrates during their synthesis (Awan *et al.,* 1999; Ritenour *et al.,* 1996). Cytokinins enhanced the cell expansion in soybean (Makarova *et al.,* 1988) and increased stem thickness while kinetin reduced shoot length but increased the fresh weight by increasing stem diameter in morning glory (Kaul & Farooq, 1994) and in okra (Chaudhry & Khan, 2000). There are also some reports which indicate that kinetin in combination with GA_3 enhanced germination and seedling growth in chick pea; it might be mediated through changes in the activities of enzymes of carbohydrate metabolism (Kaur *et al.,* 1998). Plant growth regulators (IAA, GA_3 and Cytokinins) induced a marked accumulation of protein content and carbohydrates content (Abdel-Latef, 2003; Abou Al-Hamd, 2007).

PGRs are also used to control vegetative growth thereby increasing the plant population per unit area with regard to yield (Latimer, 1991; Ouzounidou *et al.,* 2008). Flowering has been hastened or delayed by PGRs depending on species (Latimer, 1991). The effects of growth regulators for improving the growth and productivity in *Asparagus racemosus* have indicated that combination of growth regulators can improve the productivity in *Asparagus* (Vijay and Kumar, 2005). The significant increase in morphine yield of opium per plant (104%) using foliar application of GA_3 and triacontanol in combination have been reported (Khan *et al.,* 2007).

Growth regulators have tremendous effects on sex expression and flowering in various cucurbits leading to either suppression of male flowers or an increase in the number of female flowers (Al-Masoum and Al-Masri, 1999) without imposing any deleterious effect on the environment and human health. Exogenous application of plant growth regulators can alter the sex ratio and sequence if applied at the two- or four leaf stage, which is the critical stage at which the suppression or promotion of either sex is possible (Hossain *et al.,* 2006). Plant growth regulators are known to have their positive effect on growth, translocation and flowering (Crozier and Turnball, 1984; Hayat and Ahmad, 2001).

The most widely available plant growth regulator is GA_3 or Gibberellic Acid, which induces stem and internode elongation, seed germination, enzyme production during germination and fruit setting and growth (Davies, 1995). Gibberellic Acid (GA) comes under the naturally occurring growth hormone, which regulates the growth and development of plants (Jaleel *et al*., 2009). The GA are associated with various plant growth and development processes and regulate diverse activities in plants such as cell division and cell elongation, seed germination, hypocotyl elongation, shoot elongation, leaf expansion, floral initiation, uniform flowering, floral organ development, reduced time to flowering, increased flower number and size and induction of some hydrolytic enzymes in the aleurone of cereal grains (Akazawa *et al*., 1990; Matsuoka 2003; Swain and Singh, 2005; Khassawneh *et al*., 2006; Srivastava and Srivastava, 2007).

Gibberellic acid can stimulate growth by increasing cell elongation in some plant species and by increasing both cell elongation and cell division in others. They promote early flowering in different floriculture crops, increase stem length and number of flowers per plant and thereby increase yield and quality (Kazaz *et al*., 2010). The vegetative growth characteristics of gladiolus plants were improved as a result of using GA_3 (Dataram *et al.,* 2001; Kirad *et al.,* 2001; Prasad *et al*., 2001). GA_3 levels used enhanced the vegetative growth of *Zantideschia aethiopica* plants (Attia, 2004; Brooking and Cohen, 2002). The effect of Gibberellic Acid on growth, photosynthesis, enzyme activities and productivity have been well studied (Hayat and Ahmad, 2001; Saxena and Pandey, 2001; Khan and Samiullah, 2003).

In *Mentha piperita* GA_3 enhanced the fresh and dry weights, and stem length increased to a larger extent when compared with control (Gopi and Panneerselvam, 2011). Significant increase in plant height in *Abelmoschus esculentus* was noticed with GA_3 (25 and 50ppm). GA_3 (25 and 50ppm) has recorded significantly maximum LAI (Leaf Area Index) and SLW (Specific Leaf Weight) as compared to other treatments. The application of GA_3 (50ppm) recorded significantly higher chlorophyll content over all other treatments. The data on fresh yield indicated that the increase in fruit yield was significantly higher in GA_3 (25 and 50ppm) as compared to other treatments and was found lowest in control (Surendra *et al.*, 2006).

Youssef (2004) found that spraying *Sterlizia reginae* plants with GA_3 at 100 or 200ppm improved the vegetative growth parameters. In addition, Abou El-Elela (2007)

showed that spraying *Acanthus mollis* plant with GA_3 enhanced vegetative growth measurements. GA_3 also increased mobilization of starch in cotyledons by increasing amylase activity. Spraying of GA_3 @ 50ppm at four-leaf, flower and fruit initiation stage of *Luffa acutangula* significantly improved the vine length. Maximum vine length and number of branches was recorded with GA_3 @ 50ppm at four-leaf, flower and fruit initiation stage (Hilli *et al*., 2010). GA increased the total number of flowers produced per plant and decreased the number of days required for the production of 100 flowers. It was also effective in inducing enhanced pod yield, number of pods per plant, pod weight per plant and shelling percentage significantly (Verma *et al*., 2009).

Application of GA (1000 g m^{-3}) resulted in changes in leaf morphology, increase in stem elongation, leaf and internode length, plant height, and decrease in biomass content. Phenotypic changes were accompanied by decrease in contents of chlorophylls and in photosynthetic capacity. GA application resulted in higher percentage of total alkaloids accumulated in leaf, stem, and root. ^{14}C assimilate partitioning revealed that ^{14}C distribution in leaf, stem, and root of treated plants was higher than in untreated and variations were observed in contents of metabolites as sugars, amino acids, and organic acids. Capacity to utilize current fixed ^{14}C derived assimilates for alkaloid production was high in leaves but low in roots of treated plants despite higher content of ^{14}C metabolites such as sugars, amino acids, and organic acids. In spite of higher availability of metabolites, their utilization into alkaloid production is low in GA-treated roots. The study indicates that GA treatment on whole plant produced negative phenotypic response in total biomass production with positive response in the content of total alkaloids in all plant parts. But on the whole, the total production of alkaloids was decreased (Srivastav and Srivastav, 2007).

Khan and Chaudhury (2006) studied the florigenic effects of GA_3 and heavy metals in *Cucumis sativus* L. and *Momordica charantia* L. GA_3 caused precocious flowering, increase in the number of pistillate and staminate flowers in both the plants. Application of $Pb(NO_3)_2$ and $HgCl_2$ caused significant delay in flowering and reduction in number of flowers. However, when GA_3 was applied with $Pb(NO_3)_2$ and $HgCl_2$, there was less decrease in staminate and pistillate flowers, revealing the dominant effect of GA_3. It showed that the inhibitory effects of heavy metals **o**n flowering were partially restored by phytohormones.

GA_3 was even found to neutralize the ill effect of soaking treatment of *Brassica juncea* in NaCl (Afroz *et al.,* 2005). Chakrabarti and Mukherji (2002) noticed that GA_3 used to overcome the adverse effects of salinity in mungbean plants. Application of 100 mg per litre GA in *Salvia officinalis* resulted in higher essential oil content compared to control (Povh Onos, 2006). Study on goldenrod (*Solidago x hybrid)* revealed that once and twice foliar applications of 250 mg per litre of GA_3 shortened the days to flower and increased stem length, stem diameter, stem fresh weight, number of secondary inflorescences and number of stems per plant. In addition, the paclobutrazol treatments slightly retarded the days to flower and significantly reduced stem length and stem weight (Kazaz *et al*., 2010).

Effects of foliar application of growth regulators on the vegetative growth and carbohydrate accumulation in shoots and roots of *Prunus amygdalus* and *P. webbii* were studied by Mobli and Baninasab (2008). Most levels of plant growth regulators significantly enhanced seedling growth. However, GA_3 alone was most effective on stem height, leaf area, and shoot fresh and dry weights of both almond species. The thickest stems of *P. amygdalus* and *P. webbii* were obtained from the application of 100 mg per litre GA_3 followed by application of (1000 and 500) mg per litre Paclobutrazol (PBZ), respectively. In both species, PBZ significantly increased leaf chlorophyll content compared with the controls as well as with the other treatments. Application of GA_3 alone on *P. webbii* and of GA_3 followed by 100 mg per litre ethephon on *P. amygdalus* showed the highest root number, and root fresh and dry weights. High levels of soluble sugars and starch in the shoots and roots of both species were observed when GA_3 application was followed by PBZ.

Studies have shown that the use of plant growth regulators increases the secondary metabolites of medicinal plants. The influence of GA_3 on biosynthesis and accumulation of alkaloid content was recorded by various workers in *Solanum khasianum* (Gowda, 1986). The effect of Gibberellic acid (GA_3), paclobutrazol (PBZ), and *Pseudomonas fluorescens* elicitors (PF Elicitors) on the alkaloid profile variation of *Catharanthus roseus* was investigated and the alkaloid content was found to increase significantly under all the treatments (Abdul *et al.*, 2009).

GA_3 increased the plant height, number of leaves, number of shoots and leaf area, whereas maleic hydrazide and paclobutrazol reduced all these parameters compared to

control in *Polyanthus tuberosa*. Flowering was enhanced by GA_3. Spraying GA_3 at 150ppm, paclobutrazol at 1500ppm followed by maleic hydrazide 1000ppm at 30 and 60 days after planting is ideal to realize higher flower spike yield, whereas spraying GA_3 at 150ppm has realized maximum loose flower yield (Padaganur *et al.*, 2005).

Pre- and post-harvest physiology and quality responses of green pepper (*Capsicum annuum* L.) on exogenous Gibberellic acid-GA_3 (100 μM), Prohexadione-Calcium (100 mg/l), Cycocel (100 mg/l) and Ethephon (100 mg/l) applied as foliar sprays, were investigated. GA_3 exhibited better results concerning plant height and number of fruits per plant. GA_3 promoted the elongation of the first internode by 30% of the control. Improvement in *Capsicum annuum* growth and yield under GA_3 application compared to the control was observed (Ouzounidou *et al*., 2010).

GA_3 and IAA have regulatory effect to enhance the plant height, number of branches, number of leaves as compared to other plant growth regulators and control (Sarkar *et al*., 2002). Foliar spraying with GA_3 and IAA have actively increased the plant height, dry weight of plant, number of branches, number of seeds per plant, pods and seed yield per plant and unit area. However, the effects of GA_3 were greater in this regard (Marie *et al*., 2007). Similarly Rattan *et al.* (1987) reported that application of GA_3 and IAA increased the number of pods and fresh pod yield in okra. The effects of Gibberellic Acid on broccoli was studied by Wang and Yang (2008) and reported that inflorescence differentiation and curd yield was increased, but the vitamin C of curd was decreased or varied.

Youssef and Gomaa (2008) investigated the effect of GA_3 treatments (0, 100, 200 and 300ppm) on dahlia applied by three methods i.e., tubers soaking before planting, foliage spraying and tubers soaking + foliage spraying. They found that all tested treatments of GA_3 succeeded in improving the studied vegetative traits as well as leaf chemical composition determinations. The investigation was carried out to study the effect of Gibberellic Acid (GA_3) and Indole 3-acetic acid (IAA) on the growth and phytochemical composition of *Balanites aegyptiaca*.

Mostafa and Alhamd (2011) found out that the seeds of *Balanites aegyptiaca* soaked in Gibberellic Acid (GA_3) solutions (0, 50, 100, 150ppm) and Indole 3-acetic acid (IAA) solutions (1000, 2000, 3000ppm) for 14 hours showed significant increase in the germination percentage plant height, number of branches and leaves, total chlorophyll

content, dry weight and protein, carbohydrates, alkaloids, tannins and saponins. In contrast, a decrease in phenols content was found using all concentrations of both GA_3 and IAA. The concentrations of 50ppm of GA_3 and 200ppm of IAA gave the best results by increasing the growth and phytochemical compositions. The increase in seed germination percentage as a result of the exogenous application of both growth regulators was positively correlated with the decrease in total phenols. This result agrees with those of Baskin and Baskin (1998) and Araby *et al.,* 2006. Some others suggest that these phytoregulators might modulate the expression of secondary compounds of a terpenic (Ortuno *et al.,* 1993), phenolic (Garcia Puig *et al.,* 1995) and alkaloid (Cho *et al.,* 1998) nature.

GA_3 and IAA treated plants exhibited higher values of dry weight and chlorophylls content than did the control (Abdel-Latef, 2003; Afroz *et al.,* 2005; Abou Al-Hamd, 2007). Enhanced germination and seedling growth by plant growth regulators may be mediated through changes in the activities of carbohydrate metabolism enzymes (Kaur *et al.,* 2000). The increase in the dry matter due to soaking in GA_3 and IAA solution might be attributed to rapid increase in cell division, cell enlargement and accumulation of building units that accompanied by greater saccharides content than those of untreated plants (Abdel-Latef, 2003; Abdel-Latef *et al.,* 2009).

GA_3 and IAA induced a marked accumulation of protein content and carbohydrates content. This accumulation of carbohydrates due to GA_3 and IAA treatment might be linked with the efficiency of photosynthetic apparatus, which leads to increase in plant productivity and dry matter production (Azooz *et al*., 2004). Increase in protein content by plant growth regulators may be due to increase in the formation of rough endoplasmic reticulum that provides the appropriate medium for increasing polyribosome and mRNA (Kaber, 1987).

Das Gupta *et al*. (1994) recorded that foliar application of plant growth regulators like IAA and GA helped the plant to restore retardation in water content in mungbean plants subjected to water stress. Studies on seed germination and seedling growth of black gram and horse gram by Chauhan *et al*. (2009) have shown that GA_3 could overcome the adverse effects than the IAA in the seed physiological activity. The findings support the report of Chakrabarti and Mukherji (2002). Mukhtar (2008) studied the effect of GA_3, IAA and coconut milk on the growth and nutritional value of *Hibiscus sabdariffa.* Treatments

with 100ppm GA_3 and 15% coconut milk induced the greatest increase in height, chlorophyll, biochemical and some mineral element content of the plant.

Goufo *et al.* (2010) demonstrated that treatments of aromatic rice cultivars with growth regulators like Gibberellic acid, paclobutrazol, 3-indole acetic acid, and a regulator mixture consisting of paclobutrazol, proline and zinc chloride inhibited the metabolic processes associated with the formation of volatile compounds. All treatments with growth regulators resulted in reduced aroma content that affected overall flavor. Control samples were significantly higher in intensity than treated samples. GA_3 and kinetin exhibited beneficial effect in several cole crops (Chhonkar and Singh, 1963; Badawi and Sahhar, 1978).

Rana *et al.*, 2011, found that GA_3+kinetin treatment had maximum influence with respect to plant height, number of leaves and head size and hence gave the best performance in terms of yield and quality in sprouting broccoli (*Brassica oleracea* var. *italica*). GA_3 (60 mg per litre) treatment proves to be the most effective among all the treatments and required minimum days to central head formation and secondary head formation. Similar results were found by Gonzalez *et al*. (2007), Lone *et al.* (2005), Khan *et al*. (2002), Singh and Lal (2001) and Kumar and Ray (2000) in both GA_3 and kinetin, Patil *et al.* (1987), Chhonkar and Singh (1963) and Kumar *et al.* (1996). The possible reason for increase in plant frame, number of secondary heads per plant and harvest duration of sprouting broccoli plants may be due to Gibberellic Acid, which promotes vegetative growth by way of cell elongation and cell division. Both the regulators play important roles, such as Gibberellic Acid help in cell elongation, fruit growth, tissue growth and development and kinetin mainly incites cell division, delay of senescence and cell enlargement etc, so that the combinations show the best result.

Naeem *et al.* (2004) examined the effect of some growth hormones (GA_3, IAA and kinetin) on the morphology of shoot of lentil. GA_3 showed a marked elongation in the length of shoot, increase in the number of internodes and compound leaves, early flowering with higher number of floral buds. Application of IAA showed a decrease in length of shoot and number of internodes. The increase in the diameter, area and number of leaves was also observed. IAA induced branching with lush green colour of leaves, late flowering and increase in the number of floral buds. Kinetin showed inhibition in length and in the number of internodes. Inhibition was associated with a significant expansion in

diameter and an increase in area of leaves as well as their number. It showed no significant delay in flowering but number of floral buds was more as compared to control.

The combined dose of GA_3+IAA, GA_3+kinetin and GA_3+IAA+kinetin showed a significant increase in length and number of internodes as well as in the number of compound leaves. The colour of leaves was green and no branching was induced. However, the diameter of main stem showed inhibition. The dose of IAA+Kinetin showed a decrease in length and number of internodes. However, expansion in the main stem diameter and increase in the number and area of leaves was also observed. The colour of leaves was lush green with more branches as compared to control. The mixed doses of GA_3 with IAA and kinetin revealed early flowering along with non-significant increase in the number of flower buds. However, the dose of IAA+Kinetin promoted late flowering with noticeable increase in the number of floral buds (Naeem *et al.,* 2004).

Gupta *et al*., 2010, reported significant variation in growth of tuberous roots of *Coleus barbatus* due to hormonal treatments. IAA at 10^{-5}M was observed highly effective as compared to control, GA_3 and Kinetin. Application of IAA at 10^{-5}M resulted in maximum promotory effect on number of tubers, fresh weight and dry weight, followed by GA_3 at 10^{-5}M, Kinetin at 10^{-7}M as compared to control. Application of IAA, GA_3 and Kinetin resulted in more number of tubers.

Applications of growth regulators on snap bean (*Phaseolus vulgaris* L.) can alleviate salinity and water stress and can be an economic and safe alternative to environment (Torres-García *et al.,* 2009). The growth regulator used was a commercial mixture (Vitarise ®), which has as its principal components gibberellins, auxin and cytokines.

The effects of Gibberellic Acid (GA_3), along with Kinetin (K), Salicylic Acid (SA) and Ethephon were studied on growth, total flavonoid, gibberellins (GA) and salicylic acid (SA) contents of dandelion (*Taraxacum officinale).* GA_3 markedly enhanced fresh shoot weight, kinetin significantly enhanced dry root mass as compared to control. SA enhanced both shoot and root attributes, while ethephon decreased plant growth. Endogenous bioactive GA_1 and GA_4 content and SA content enhanced with the application of GA_3, SA and kinetin, but declined with ethephon. The flavonoid content of dandelion significantly increased with SA treatment, but was not altered with the application of other PGRs (Kim *et al.,* 2009).

IAA is the major auxin involved in many of the physiological processes in plants. Foliar application of cowpea plants with Indole Acetic Acid (IAA) at three concentrations (12.5, 25 & 50ppm) induced increments of the plants height, fresh and dry weights, number of branches and number of leaves per plant as well as yield components (pods per plant, seeds per pod, weight of pod, weight of seeds per plant and weight of seeds per feddan). In addition, IAA can be used for overcoming the stress induced by drought and it can be used in combination with organic manures either chicken or farmyard, to reclaim arid soils as reported by El-Bassiouny (2001). The increase in growth and yield of *Artemisia annua* due to application of IAA has been obtained (Yaseen and Tajuddin, 1998).

Application of IAA at the rate of 10^{-5}M increased grain yield and biological yield in wheat (Arif *et al*., 2001). Similarly, Zahir *et al*., (2000) reported up to 50% increase in fresh biomass of soybean by the application of L-tryptophan (precursor of IAA). Indole 3-acetic acid (IAA) increased growth and yield of black seeds as found by Hussien *et al.,* 2003. Ashraf *et al.* (2006) concluded that IAA is successful in enhancing the plant growth and yield of barley cultivars and alleviated the adverse effect of water stress. IAA treatments of cowpea at the rate of 25 and 50 mg/l increased number of leaves, shoot dry weight and number of produced flowers per plant. Meanwhile 50 and 100mg IAA significantly decreased the number of flowers abscised from cowpea plant. IAA at 25 and 50mg/l significantly increased the number and weight of pods and seeds per plant. Endogenous IAA, Gibberellins and Cytokinin increased during flowering and at abscission time, however, Abscisic Acid content was decreased by all applied concentrations of IAA (El-Saeid *et al*., 2010).

IAA irrespective of concentrations in combination with boron and zinc at flowering and podding stages was found most effective in prevention of flower and premature abscission and acceleration of assimilate translocation as well, while foliar application of the same at flowering or pod initiation, individually, seems to alter the growth parameters or yield contributing traits partially (Tekale *et al*., 2009). Gudhate *et al*., 2009 has reported the effect of plant growth regulators for improving andrographolide in *Andrographis paniculata.* The treatments of IAA and NAA (50mg/l) were found to be the most effective for improving whole plant biomass as well as andrographolide content. The same treatments were also effective for improving fresh and dry biomass of the whole plant. This suggests that the improvement in quality as well as quantity can be achieved

with the use of foliar application of PGRs, which can be a good approach of secondary metabolite improvement for growers. Similar results were reported in *Solanum nigrum* by Bhatt *et al*., 1983. They noted positive influence of IAA on alkaloid production. Reports in *Solanum jaminoides* (Sahoo *et al*., 1999) *Solanum khasianum* (Bores *et al*., 2001), had also shown positive influence of IAA on secondary metabolite production.

Cytokinins have been implicated to control many developmental processes and environmental responses of plants, including leaf senescence, apical dominance, chloroplast development and regulation of cell division (Hutchison & Kaber, 2002). Kinetin, a synthetic Cytokinin, play important role in plant growth and development. It stimulates leaf expansion, development of reproductive organs and delays senescence (Mock, 1994). Kinetin improves the export of assimilates from source organs and regulate source-sink relations (Fritsch and Hens, 2000). Cytokinins are involved in various processes in the growth and development of plants (Takei *et al*., 2002). These effects are due to interactions with other plant hormones and environmental signals (Hare *et al*., 1997).

Cytokinins have been shown to participate in the regulation of numerous aspects of plant development including initiation of buds, flowering, abscission and yield (Zibeline *et al*., 1985; Morris *et al*., 1990 and Dubing *et al*., 1996). Results obtained by several investigators emphasis the role of Cytokinins in retardation of abscission process (Khalil *et al.,* 2006; Okelana and Adedipe, 1982; Mauk *et al.,* 1986) with respect to lentil, cowpea and citrus plants, respectively. Endogenous IAA, Gibberellins, Cytokinins increased during flowering and at abscission time, however, ABA decreased by all applied concentrations of kinetin (Khalil *et al*., 1990).

Kinetin treatments decreased plant height in *Lens culineris* (Khalil *et al*., 2006), cowpea (Khalil and Mandurah, 1990) and tomato (Almugadam, 1997). However they induced significant increase in the number of leaves, branches, shoot dry weight and number of produced flowers per plant in lentil (Khalil *et al.,* 2006), pea (Griga *et al*., 1984) and alfalfa (Tomkins and Hall, 1991). These results may be attributed to the high level of endogenous Cytokinins. In *Salvia sclarea* L., maximum height and leaf numbers were observed after application of kinetin 10 µ/l and IAA 50 µ /l respectively (Silva *et al.*, 2006). Kinetin reduced stem length of cowpea shoots. On the other hand, it exhibited

simulative effect when applied at concentrations 10 and 20 mg/1 on number of internodes and dry weight of shoots (Khalil and Mandurah, 1990).

Matthysee and Scott, 1985 stated that Cytokinins serve as a carrier from the root apex as it regulates the growth of lateral roots and lateral branches of the shoots. Kinetin treatments increased the dry weight, flower potential and number and weight of pods and seeds per plant of lentil shoot though they decreased stem length and the percentage of abscised flowers (Khalil *et al*., 2006). Das *et al*. (2002) reported that foliar application of kinetin increased chlorophyll content and leaf yield of mulberry. Increase in the number of flowers by Cytokinins was recorded in other plants such as beans and soybean (Dubing and Westgate, 1996; Lynas, 1981; Mansour *et al.,* 1994). Increase of yield of some legumes by Cytokinins (kinetin or benzyladenine) was reported by Zhlobak (1986) on pea, Salem (1989) on soybean, Khalil and Mandurah (1990) on cowpea. Increase in seed yield by kinetin application was reported by Fatima and Bano (1998) in soyabean and Faizanullah *et al.* (2010) in linseed.

Zahir *et al.* (2007) reported increase in growth and yield of wheat in kinetin-blended N-enriched compost. GA-blended and IAA-blended compost showed non-significant improvement. Significant increase in 1000-grain weight and protein quality of wheat has been reported in response to kinetin application (Wierzbowska and Nowak, 1998). Kinetin increased both shoot apex development and the final ear size in barley. The weights of the main shoot and the whole plant also increased (Ruckenbauer and Kirby, 1973). Wang *et al.* (2009) studied the effect of different concentration of cytokinin on broccoli and found that it increases ball-flower yield and plants economic coefficient, but weakened ball flower quality. In mint (*Mentha arvensis*) the use of 200ppm of kinetin resulted in an increase of biomass and essential oil yield (Farooqi *et al*., 2003). Ullah and Bano (2011) reported that kinetin treatment was highly effective in increasing achene yield, 100 achene weight and oil refractive index of safflower (*Carthamus tinctorius* L.).

Seed priming with optimal concentration of the Cytokinins has been shown to be beneficial to germination, growth and yield of some crop species grown under saline conditions (Kaur *et al*., 2002). Some other studies have also shown that kinetin induced salt tolerance in wheat (Iqbal and Ashraf 2005). It has been reported that kinetin treatment improved the water status of wheat plants grown in high salinity (Gadallah, 1999). Foliar application of kinetin and IAA, especially at 2 mM, counteracted some of the salt induced

adverse effects on maize plants by enhancing essential inorganic nutrients as well as by maintaining membrane permeability, but, in combination, they did not ameliorate the adverse effects of salinity (Kaya *et al*., 2010). Kinetin application helped wheat plants to grow successfully in the areas subjected to combined effects of salinity and oxygen deficiency, such as in salt marshes (Gadallah, 1999).

Plant growth retardants generally have the greatest effects on expanding or elongating cells, where inhibition of Gibberellins synthesis rapidly causes reduction in stem elongation and leaf expansion (Tanimoto, 1987; Leclerc *et al*., 2006) and reduce cell division and cell elongation (Halevy, 1986; Rademacher, 1993; Boldt, 2008). Therefore, they are commonly used in floriculture industry for height control (Bailey and Whipker, 1998; Pasian, 1999; Hayashi *et al.*, 2001; Karlovic *et al.* 2004). Growth retardants at the same time increase the number of lateral shoots, resulting in a larger number of inflorescences (Whealy *et al.*, 1988; Keever and Foster, 1989).

Many growth retardants are known to reduce the internodal length, reducing the plant height (Detotale *et al.,* 1994) and there by influence the source sink relationship and stimulate the translocation of photosynthates towards sink. Application of growth retardants may also enhance the chlorophyll content of leaves which helps to increase the functional life of the source for a longer period leading to improved partitioning efficiency and increased productivity (Kashid *et al*., 2010).

Maleic Hydrazide (MH) is an anti-auxin growth regulator and acts as a growth retardant, known to cause inhibition of seedling growth by inhibiting mitotic cell division in plants (Zukel 1950). Growth inhibitory effects of MH has been reported by Schoene and Hoffman (1949), Aurbey and Naylor (1950), Sircar and Ray (1962), Larry (1969) and Kumar and Pal (2004) in various plant species. These reports indicated that the growth inhibitory effect was dose dependent and more conspicuous at seedling stage affecting both root and shoot growth.

Thappa *et al.* (2011) studied the floral and yield traits in cucumber by a combined application of 100ppm maleic hydrazide and 100ppm Ethephon. This treatment induced early development, maximized the sex ratio with regard to yield and was comparatively helpful in reducing plant expansion. Sen and Naik (1977) noticed maximum reduction in growth of chrysanthemum with maleic hydrazide followed by cycocel. Spraying of MH at 500ppm reduced the plant height but increased the number of lateral branches and leaves

compared to control in chrysanthemum (Beach and Leopold, 1963), carnation (Dubey, 1972), china aster (Reddy and Sulladmath, 1983) marigold (Lal and Mishra, 1986, Singh, 2004). Further, Singh reported increase in leaf area index. Suppression of growth by spraying MH in chrysanthemum was observed.

Dry weight of shoot was increased with 500ppm of MH but it was reduced with higher concentrations (Shanmugam *et al*., 1973). Narayan Reddy (1978) and Aswath *et al.* (1994) noticed that foliar application of MH at 750 and 1000ppm resulted in significant reduction in internodal length and increase in number of leaves and branches in China aster. Further, it was noticed that all the concentrations reduced the diameter of flowers in winter as well as in summer crops. Flower numbers were increased by spraying MH while flowering was delayed by 8 to 18 days depending on the concentration in chrysanthemum (Sen and Maharana (1972), marigold (Parmar and Singh (1983). Similar results were obtained in China aster (Reddy and Sulladmath, 1983).

Jitendrakumar and Sanjeevkumar (2004) reported that number of days taken to first flower bud opening was more with higher concentration of MH (700ppm) compared to lower concentrations (250ppm). Further, they reported that more number of flowers per plant was recorded with 250ppm of MH and less with 700ppm of MH in balsam. Spraying of MH at 500ppm significantly increased the germination percentage and vigour index in gaillardia but it did not influence the shoot and root length of the seedling (Hugar, 1977). Doddagoudar *et al.* (2004) and Singh (2004) reported increased seed weight, germinability, shoot and root length, seedling vigour index and seedling dry weight with MH 500ppm spray in China aster and marigold respectively.

The growth retardant succinic acid 2, 2-dimethylhydrazide (SADH) reduces both vegetative and fruit growth of apples (Batjer *et al.*, 1964) and induces similar growth responses in many other plant species (Cathey, 1964). It is used commercially on apples to increase fruit firmness and red colour; to delay development of water core; and to decrease fruit size and vegetative growth on young vigorous trees (Bartram, 1960).

Williams and Stahly (1970) observed that fruit from Red Delicious apple trees treated with the growth retardant succinic acid 2, 2-dimethylhydrazide contained more N-malonyl-D-tryptophan (MT) than control fruit. This cannot be attributed to an advance in fruit maturity since some experiments have shown that SADH-treated fruits ripen more slowly than control fruits (Williams, 1964; Looney, 1968). Thus the increase in MT

appears to be a direct result of SADH treatment. Further when SADH and tryptophan were injected into immature fruits, more N-methyl-D-tryptophan was produced than when DL-tryptophan was injected alone. This suggests that SADH may control fruit and vegetative growth by interfering with auxin production.

SADH treatments of eight apple cultivars induced reductions in catalase activity and iso-peroxidase spectrum during growing season which were associated with reduced vegetative growth and enhanced fruit bud formation. The winter hardiness of cultivars which reacted strongly to SADH treatments was improved (Badescu *et al*., 1972). The application of SADH followed by Ethephon, brought 95% of the berries to full ripeness, reduced the length of the harvest period by approximately one week, and had no significant effect on the size of the berries (Dekajos, 1976). SADH was found to delay ripening of apples (Looney, 1967), advance maturity and improve fresh color of peaches and sour cherries (Unrath *et al*., 1969).

Abscisic Acid (ABA) is a plant growth regulator which regulates processes of embryo maturation, seed maturation and dormancy, seed development, seed germination, stomatal opening, root development, floral transition and tolerance to biotic and abiotic environmental stresses (Giraudat *et al.*, 1994; Mahovachi *et al*., 2005; Beaudoin *et al*., 2000). ABA is an important regulator in many aspects of plant growth and development, and is pivotal for stress resistance. Abscisic Acid (ABA) has been shown to mediate many physiological and developmental processes throughout the life cycle of plants including responses of plants to environmental stresses. Its level increase as a result of stresses including drought, salinity and cold stresses that involved cellular water stress (Khadri *et al*., 2006).

ABA is known to act as a major signalling molecule involved in the response of plants to drought stress. Stress-related responses induced by ABA often occur earlier than the change of plant water status during soil drying and thereby constitute the first line of defence as soil water deficits are encountered (Liu *et al*., 2005). The hormone triggers stomatal closure to limit water loss through transpiration, thus limiting photosynthetic CO_2 assimilate, as well as mobilizes a battery of genes that presumably serve to protect the cells from the ensuing oxidative damage in prolonged stress (Wasilewska *et al.,* 2008). These physiological and biochemical changes have been proposed to reduce the deleterious effects induced by water stress.

Many studies have shown that ABA is able to induce changes including synthesis of stress proteins, proline, sugar alcohols, soluble carbohydrates and glycine betaine which may involve in stress tolerance (Bagniewska-Zadworna *et al.,* 2007). Furthermore, application of exogenous ABA even triggers more sugar accumulation, but decreases starch content in *Polypodium vulgare* (Bagniewska-Zadworna *et al.,* 2007). Exogenous ABA was also reported to reduce photosynthetic rate, stomatal conductance and transpiration rate in cotton (Pandey *et al.,* 2003). Exogenous ABA helps the plants to better maintain cellular water level, also reported in chickpea, *Ilex paraguariensis* and *Polypodium vulgaris* (Sansberro *et al.,* 2004; Bagniewska-Zadworna *et al.,* 2007, Kumar *et al.,* 2008).

In addition, application of ABA are known to affect plant growth and development, mimicking the effects of water stress, thereby helping plants to better survive stress conditions (Farooq *et al.,* 2009 a). Exogenous ABA was shown to help the plants to maintain their relative water content and enhanced sugar accumulation under drought stress (Wattana, 2011). Exogenous ABA application decreased Na^+ accumulation and increased K^+: Na^+ ratio in sorghum and rice shoot and also in leaves (Amzallag *et al*., 1990). ABA reduces transpiration by closing stomata and thus leads to reduce ion uptake in plant (Yeo *et al*., 1985). In view of this information, Ashraf & Foolad (2005; 2007) proposed that plant growth regulators, compatible solutes, antioxidants or inorganic salt can be exogenously applied as a foliar spray, or through seed priming to induce stress tolerance.

Mengual *et al.* (2003) studied the effectiveness of different plant growth regulators such as Abscisic Acid, Jasmonic Acid and 8'-methylene methyl abscissate in protecting citrus from salt-induced damage. They reported that ABA plays a role in modifying citrus physiological behaviour in response to salinity. Pre-treatments with ABA effectively increased salt tolerance in crops such as tobacco and barley and forest species such as *Pinus banksiana* (Larosa *et al.*, 1987; Popova *et al.*, 1995; Rajasekaran and Blake, 1999). Astacio and Iersel (2011) demonstrated in tomatoes (*Solanum lycopersicum*) that ABA drenches rapidly close stomata, limit transpirational water loss, and can extend the shelf life of retail plants by up to 8 days, which exemplifies its potential as a commercially applied plant growth regulator. Higher ABA concentrations resulted in less water use by both well-watered and un-watered plants. Negative side effects of the ABA application were rate-dependent chlorosis of the lower leaves followed by leaf abscission.

Exogenous ABA significantly decreased yield of green and red lettuces. Total phenolic and total anthocyanin contents in red lettuce treated with ABA were significantly higher than in controls, whereas no significant differences were observed in green lettuce. ABA significantly induced the accumulation of chlorophyll b and total carotenoids in lettuces and it elevated the content of individual phytochemicals in red lettuces (Li *et al.*, 2010). Exogenous application of 10 µM ABA leads to swelling, root hair formation and initiation of lateral root primodia in the tips of young, seminal rice roots. ABA treatment significantly increased 2, 3, 5-triphenyl tetrazolium chloride (TTC) reductase ability in the root tips and the exudation rate of xylem sap (Chen *et al.*, 2006).

Exogenous ABA increased shoot dry weight and maintained a high concentration of photosynthetic pigments for a longer period of time during grain growth and maturation. Although ABA applications increased stomatal closure immediately after its application, the longer-term effect was to allow for a greater ostiolar opening of the stomatal pore which resulted in increased conductance of gases and water vapour. ABA also improved the transport of photoassimilates from the leaves and stem to the developing grains, that is, it effectively increased the sink strength of the grains. This correlated with a yield increase without significantly changing the protein quality in the grains (Travaglia *et al.*, 2010).

Exogenous ABA and proline treatment to dormant seeds of *Laurus nobilis* L. significantly affected physiological and biochemical traits of 6-month-old seedlings, by inducing the responses against drought, in a well watered condition (Aktas *et al.*, 2007). ABA is partly responsible for the differential response of root and shoots growth to dry soils. In dry soil it maintains root growth and inhibits shoot growth. However, when applied to well-watered plants, it usually inhibits root and shoot growth, showing that plants in dry soil respond quite differently from well-watered plants. ABA affects the rate of cell expansion in plants in dry soils: it maintains cell expansion in roots and inhibits that in leaves (Munns and Sharp, 1993). ABA and CCC pre-soaking treatments partially alleviated the inhibitory effect of drought in *Vigna radiata* and increased the endogenous levels of phytohormones GA and IAA (Farooq and Bano, 2006). DIZ and ABA treatments increased the fresh weight, dry weight, root growth, total chlorophyll, protein and amino acid content, while it decreased the stem length (Gopi and Panneerselvam, 2011).