Chapter – I

Introduction

1.1 General Introduction

A complete understanding of medicinal plants involves a number of factors like botany, chemistry, genetics, quality control and pharmacology. In addition there is a large wealth of knowledge in the medicinal and other properties of plants from generation to generation by the tribal societies. *Tricosanthes cucumerina* L. is a well known plant, the fruit of which is mainly consumed as a vegetable. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called as snake gourd, viper gourd, snake tomato or long tomato. The fruit is usually consumed as a vegetable due to its good nutritional value. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. It has a prominent place in alternative systems of medicine like Ayurveda and Siddha due to its various pharmacological activities like antidiabetic, hepatoprotective, cytotoxic, anti inflammatory, larvicidal effects. The regional names of snake gourd or snake tomato is called in Bengali as Chichinga/ Chichinge, in Telugu as potlakaaya, in Tamil as pudalankaai, in Kannada as aduvalakaayi, in Malayalam as padavalanga.

Tricosanthes cucumerina L. falls under the scientific classification of

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Mangnoliopsida
Order	:	Curcubitales
Family	:	Curcubitaceae
Genus	:	*Trichosanthes*
Species	:	*cucumerina* L.

1.2 Description

Tricosanthes cucumerina L. is a monoecious annual herb climbing by 2–3-branched tendrils upto 5 to 6 meters high or less. The stems are slender, green, 4-angled, somewhat hairy, and faintly disagreeable in odor. The roots are somewhat

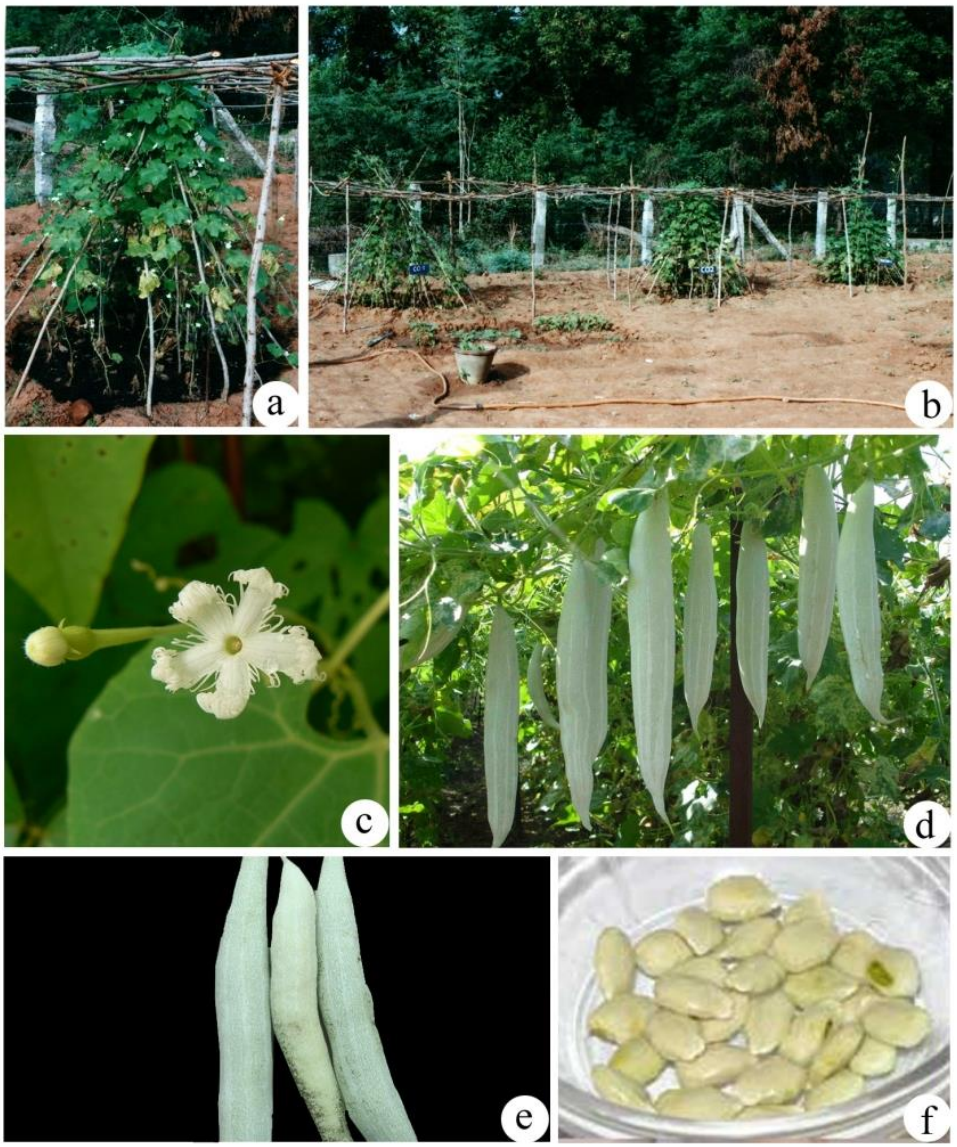
tuberous and whitish. The leaves are alternate, simple with no stipules. Leaves are scabrid hairy on both surfaces, rounded in outline, 7 to 14 centimeters long and broad, and 3 or 5-lobed, the lobes being broad, rounded or obtuse, and the sinuses broad or narrow and rounded. The base is broadly heart-shaped. The staminate inflorescences are long-peduncled and axillary, with six to fifteen flowers. Flowers are unisexual, regular, and white in colour with green and hairy calyx. Corolla is tubular in with lobes fringed and hair like outgrowths. The male flowers are many-flowered with axillary racemes on 10– 30 cm long peduncles. They are with 3 stamens but the female flowers are solitary and sessile with inferior, single celled ovary, long and with hairy stigmas. Fruits are very slender, long and cylindrical berry, often twisted, greenish-white when immature, dark red when mature. The seeds are half-ellipsoid, somewhat compressed, undulate, hard, rugose, nearly one centimeter long, greyish-brown, sculptured, margin undulate and imbedded in a soft foetid with red pulp.

1.3 Origin and distribution

The genus *Trichosanthes* is native to Southern and Eastern Asia, Australia and Islands of the western Pacific. *T. cucumerina* L. is found wild throughout these areas. It was probably domesticated in ancient times in India. It is grown as a minor vegetable in many countries of tropical Asia. It is locally grown as a vegetable in home gardens in Africa. Commercial growers around big cities in East Africa occasionally grow cultivars of snake gourd imported from India for people of Indian origin. It is also reported from India through Malaya to tropical Australia. *T. cucumerina* L. is a newly introduced crop of increasing importance in several parts of Africa, including Ghana and Nigeria. The genus *Trichosanthes* comprises about 100 species, of which a few have been domesticated in Asia, snake gourd being the most important. Two varieties are distinguished within *T. cucumerina* L. They are the wild var. *cucumerina* occurring from India, Sri Lanka and China, through South-East Asia, to northern Australia, and the cultivated var. *anguina* (L.). Only traditional landraces of *T. cucumerina* L. are used in West and Central Africa, whereas improved cultivars from India are grown in East Africa. It is distributed in temperate Asian regions like china, tropical regions of Bangladesh, India, Nepal, Pakistan Sri Lanka, Myanmar; Vietnam, Indonesia; Malaysia; Philippines, in Australia it is found in Northern Territory, Queens land and in Western Australia.

Plate - 1

Trichosanthes cucumerina L.



a & b. Field Plant
c . Flower
d. Harvesting plant
e. Fruits
f. Seeds

1.4 Commercial Varieties of Snake Gourd:

Below are some of the commercial varieties of Snake Gourd cultivated in India CO-1, CO-2, PKM-1, MDU-1, PLR (SG) -1, and PLR -2.

1.5 Nutritional Value of Snake Gourd

Snake Gourd contains a rich variety of nutrients, vitamins, and minerals that are essential for human health, including significant levels of dietary fiber, a small number of calories, and high levels of protein. In terms of vitamins, snake gourd possess vitamin A, vitamin B, vitamin C, as well as manganese, magnesium, calcium, iron, potassium, and iodine. Some of the most important health benefits of snake gourd include its ability to improve the strength of the immune system, reduce fevers, detoxify the body, improve the digestive processes of the body, increase hydration in the body, treat diabetes, boost the strength and quality of the hair, and aid in weight loss.

1.6 Edible Uses

- Because of its nutritional benefits and soothing taste, the vegetable is used in various curry preparations.
- Snake gourd alongside celery and lentils makes for an essential ingredient in several traditional Indian soups or stew preparations like kootu.
- Chopped snake gourd along with capsicum and tomato makes for a nutritional salad dish.
- Due to their cucumber-like taste and texture, they are also made into pickles.
- The vegetable is stir-fried with urad dal (black gram lentils), red chilies and curry leaves to make traditional Indian dishes like snake gourd poriyal.
- Because of its soft texture, it is sauteed into a paste (chutney) with other ingredients like lentils, sesame and cumin seeds to prepare thogayal, a South Indian dish.
- Fritters prepared by dipping sliced snake gourd into a paste of besan (gram flour) and rice flour accounts for a mouth-watering snack.
- The red pulp of ripe snake gourd is an alternative for tomato in many dishes.
- Baked or sauteed snake gourd serves as a tasty side dish.

1.7 Nutritional data

Given below is the nutritional information for 100 grams snake gourd:

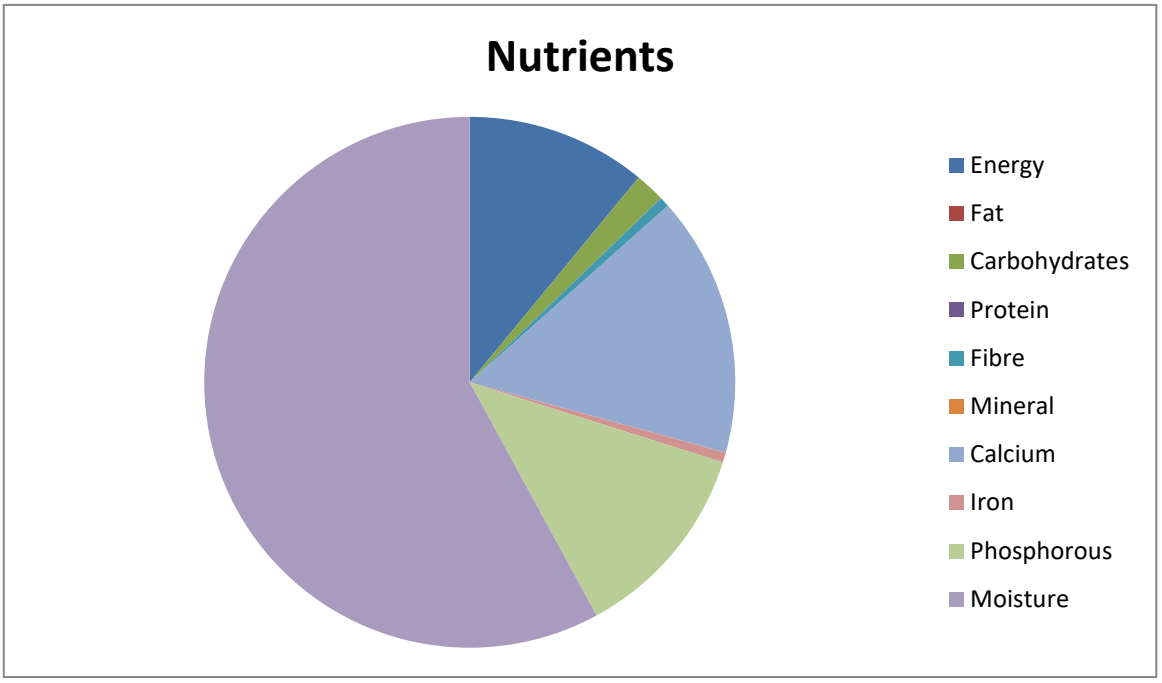


Fig. 1 The nutritional information for 100 grams snake gourd

1.8 Medicinal Uses

- Because of its hepatoprotective properties, an infusion of snake gourd leaves and coriander seeds act as an effect home remedy in fighting jaundice since it is said to lower the bilirubin levels.
- External application of snake gourd sap helps in treating dandruff and alopecia.
- The concoction made from snake gourd leaves, honey, and coriander, acts as a febrifuge as it has a cooling effect helping to reduce bilious or intestinal fever.
- The juice of its leaves acts as a mild laxative, thereby cleansing the bowels and eliminating constipation. It also induces vomiting to purge one's stomach in case of poisoning.

- When suffering from a severe cough and cold, the snake gourd extract may be used as an expectorant, helping to dissolve pus as well as phlegm in the respiratory tract.

- While small quantities of the low-calorie vegetable will prove beneficial for both the mother and the fetus, care must be taken not to consume high amounts of snake guard so as to avoid any damage to the fetus.

1.8.1 Established scientific uses Anti-inflammatory

Kolte *et al.,* (2017) have investigated that with hot aqueous extract of root tubers of *T. cucumerina* L. have investigated against carrageenin induced mouse's hind paw oedema and it exhibited significant anti-inflammatory activity.

1.8.2 Cytotoxic activity

Kongtun *et al.,* (1999) tested cytotoxicity against four human breast cancer cell lines, lung cancer cell lines and one colon cancer cell line with the root extract of *T. cucumerina* L. and the fruit juice. The root extract inhibited more strongly than the fruit juice.

1.8.3 Hypoglycaemic activity

Kar *et al.,* (2003) showed significant blood glucose lowering activity in alloxan diabetic albino rats with crude ethanolic extract of *T. cucumerina* L.

1.8.4 Larvicidal efficacy

Rahuman *et al.,* (2011) showed moderate larvicidal effects using the acetone extract of leaves of *T. cucumerina* L.

1.5.5 Hepatoprotective activity

Sathesh Kumar *et al.,* (2009) found that the methanolic extract of the whole plant of *Tricosanthes cucumerina* L. showed good hepatoprotective activity against carbon tetrachloride induced heapatotoxicity.

1.8.6 Anti-fertility activity

Devendra Kage, *et al* (2008) showed the anti- ovulatory activity of ethanol extract of whole plant of *Trichosanthes cucumerina* L. var. *cucumerina* in female albino rats.

1.9 Current Status of Snake Gourd *in vitro* Regeneration

Trichosanthes cucumerina L. var. *cucumerina* belongs to Cucurbitaceae and is distributed in throughout India, Bangladesh, Sri Lanka, Burma, Malaysia, Australia. It is a perennial climber with an attractive white flower. It is highly bitter in taste which may be supposed to contain medicinal properties (Choudhary 2003) for Skin disease (Chopra *et al.,* 1969). Early Several other protocols for plant regeneration via direct and indirect organogenesis have been developed from different snake gourd tissues. In those protocols, the regeneration efficiency has been reported to be affected by different factors, such as combination of growth regulators, explant type and genotype. Most of the organogenic systems reported are based on supplementing culture media with auxins and cytokinins, either alone (Kamat and Rao, 1978; Matsuoka and Hinata, 1979; Alicchio *et al.,* 1982; Gleddie *et al.,* 1983; Sharma and Rajam, 1995a) The seed posses anthelmintic and antifibrile properties the seeds are haemoagglutinating (Chakravarty 2010) Trichosanthin is an antiviral protein purified from the root of *T. kirilowii* Maxim. It is an active component of Chinese medicine and is still being used in midterm abortion and to treat carcinoma. Karasurin is another new abortificient protein isolated from root of *T. kirilowii* (Shunsuke *et al.,* 1991). Appetizer, laxative, aphrodisiac and blood purifier (Shivarajan and Indira 1994) The aqueous extract of root exhibited significant anti-inflammatory (Kolte *et al.,* 1996). The species belong to genus *Trichosanthes* are considered as the future plants of Cucurbitaceae (http://www.pfaf.org/index.html 1996.). Hence It is used in various treatments as a cordiotonic, antipyretic, antiperiodic, useful for intestinal worms and leaf juice rubbed over the liver in remittent fever (Kirtikar and Basu 2000). Trichosanthin shows inhibition of human immunodeficiency virus (HIV) because of its ribosome inactivating activity (Jian-Hua Wang *et al.,* 2003). Seed is a good source of nutrients (Oloyede and Adebroye 2005). Root is used to cure bronchitis, headache and boils. Leaves, for biliousness, emetic, externally applied over bald patches of alopecia to reduce congestion on congestive cardiac failure (Pullaih 2006). Isolation and

characterization of galactose specific lactin from the seed. It is used as one of the important ingredients in 16 commercially available herbal products in India.

Biotechnology has opened up new vistas for crop improvement. Biotechnological tools like *in vitro* propagation, genetic engineering and molecular biology has helped over coming constraints of conventional breeding, and identification and introduction of useful genes that confer resistance to pests and diseases, and tolerance to abiotic stresses in snake gourd. Although some developments in the field of biotechnological applications have taken place, the full potential is yet to be exploited for improvement of snake gourd. Plant regeneration studies have contributed much to the crop, as there are extensive studies on regeneration via organogenesis and somatic embryogenesis. The fundamental aspects of plant regeneration have been addressed by many studies and considerable information has been gathered at the cellular and molecular level. The understanding of specific metabolic pathways directly or indirectly involved in plant morphogenesis has helped in understanding and improving the regeneration potential of snake gourd genotypes. Improvement of organogenesis and somatic embryogenesis by manipulating the polyamines metabolism has opened new vistas not only in snake gourd tissue culture but also tissue culture of other crops. Further, the studies on differential display of transcripts during somatic embryogenesis hold better hope in understanding the molecular mechanisms that control the process of somatic embryogenesis.

1.9.1 Applications

Plant tissue culture is used widely in the plant sciences, forestry, and in horticulture. Applications include:

- The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.

- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants.
- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.

1.10 GENE TRANSFER

Generally, genetic transformation in plants has been achieved by particle bombardment, electroporation and Agrobacterium-mediated transformation. The Agrobacterium technique is most commonly used for plant transformation. *Agrobacterium tumefaciens* is infected through a wound of plant and forms a tumor, but these responses happen by bacteria internal Ti plasmid, and it is DNA fragment called T-DNA is transferred into a nucleus of a plant cell while a plant is infected, and to be happened (Ream, 1989). After the insertion of a foreign gene succeeds in a vegetable cell by Fraley *et al.*, (1986) first, gene transformation has been accomplished in plants, implicating enormous potentials to be applied to most species.

1.11 NEED FOR THE STUDY

In vitro culture based selection methods and genetic transformations through *Agrobacterium* strains were widely used for the genetic manipulation of plants. *In vitro* techniques together with genetic manipulation are applied to improve the crop plants. Plant tissue culture techniques have been alternatively proposed as a tool for propagation in plant breeding techniques of useful genotypes or as a source of novel genetic variability to be exploited in selection programme.

With the above said importance, this research work was designed to regenerate the transgenic plantlets with fungal disease resistant gene with the following objectives.

- To standardize the simple and reproducible techniques for micropropagation from shoot tip and node explants.
- To regenerate the plantlets by organogenesis through callus cultures from leaf and inter node explants.
- To standardize the somatic embryogenesis protocols through direct and indirect methods using leaf explant.
- To regenerate the transgenic plants by preliminary transformation studies using the primary gene construct LBA 4404 carrying the *gus* marker gene and *npt* II reporter gene by using an efficient regeneration protocol.
- Cloning and mobilization of *Theobroma cacao* Chitinase gene to a binary vector (pBinAR) and to regenerate the transgenic plants in *T. cucumerina* L. with *Theobroma cacao* Chitinase gene (*Chi* I) by using an efficient regeneration protocol for improved tolerance to fungal diseases.