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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(9): 2198-2208

www.thepharmajournal.com
Received: 22-07-2023
Accepted: 20-08-2023

Gita R Chaudhari
COH, Anand Agricultural University, Anand, Gujarat, India

Krishna Prakash
ICAR-Indian Agricultural Research Institute, Jharkhand, India

Sheetal R Patel
Regional Research Station, NAU, Vyara, Gujarat, India

Divya Chavda
Anand Agricultural University, Anand, Gujarat, India

Neelam Shekhawat
ICAR- National Bureau of Plant Genetic Resources, Regional Station, Jodhpur, Rajasthan, India

Sridhar Ragi
ICAR- Indian Agricultural Research Institute, New Delhi, India

Supritha Raj DS
University of Agricultural Sciences, Dharwad, Karnataka, India

Corresponding Author:
Gita R Chaudhari
COH, Anand Agricultural University, Anand, Gujarat, India

Secondary metabolites production in plant tissue culture

Gita R Chaudhari, Krishna Prakash, Sheetal R Patel, Divya Chavda, Neelam Shekhawat, Sridhar Ragi and Supritha Raj DS

DOI: https://doi.org/10.22271/tpi.2023.v12.i9Sac.23144

Abstract
Plant cell culture is thought to hold the promise of creating practical plant products without the need for conventional agriculture and all of its associated issues and uncertainties. Therefore, it is a tempting idea to produce beneficial secondary metabolites in sizable bioreactors situated in the consuming nation. Additional advantages of such processes included controlled production according to demand and a reduced and requirement. Plant tissue cultures that produce secondary metabolites from therapeutic plants.

Keywords: Secondary metabolites, tissue culture, active compounds

Introduction
Although the advantages mentioned above, there are still a number of issues that need to be resolved before this technology may be widely used to produce valuable plant secondary metabolites. Another concern is whether or not these methods are economically viable, and this can be covered in a different section.

As product secretion is not widespread, the large metabolite yields reported in bacteria that secrete product (thereby reducing product inhibition of biosynthesis) cannot be predicted. The vacuole is the primary location of product accumulation. Plant cell membrane permeabilization is the subject of continuing research, which may help to alleviate product inhibitory restrictions by facilitating leakage to extracellular media. This might aid in lowering production costs if it also allowed for the re utilization of the biomass (for instance, through immobilization). Plant cells have a benefit over microbial cultures as a whole because they require less aeration. Despite media costs are far lower compared to those of cells from animals cultures, it is still important to take into account the high expense of operating a vessel for fermentation over a period of several weeks.

Secondary Metabolites
Chemical substances known as phytochemicals together refer to the secondary metabolites that plants create. Chemical substances known as secondary metabolites are those that do not take part in plant metabolism. However, these substances play a role in pollination, stress resistance, and disease resistance (from fungi, bacteria, viruses, and pests). The stressors [drought, cold, temperature, etc.] could be biotic or abiotic. Alkaloids, steroids, terpenoids, essential oils, flavors, fragrances, colors, and pigments are a few examples of secondary metabolites.

Application of secondary metabolites
Numerous industrial products utilize secondary metabolites; among these are

- Nutraceuticals
- Textile Industry
- Cosmeceuticals
- Pharmaceuticals
- Perfume Industry, etc.

Terpenoids, Steroids and steroids are used in 25% of the prescribed drugs, and almost all cancer drugs are sourced from plants. Colours and fragrances are used in textile and food industry. Some of the plant species that produce the secondary metabolites of great industrial importance.

Production of secondary metabolites

The process of invitro culture of cells for the large scale production of secondary metabolite is complex, and involves the following aspects

- Developing Mother Culture
- Selection of cell lines for high yield of secondary metabolites.
- Large scale cultivation of plant cells
- Medium composition and effect of nutrients
- Elicitor induced production of secondary metabolites
- Effect of environmental factors
- Biotransformation using plant cell culture
- Secondary metabolites and analysis

Developing Mother Culture

The culture, which is produced, first is called mother culture. Different cultures has been developed like callus, multiple shoots, roots, etc among these which of the culture can be produced easily, will be selected for further culturing.

The callus can be developed from explant or from a mother culture, which is already developed. Different types of callus can be obtained like Nodular Callus, Friable Callus Pigment Callus, Embryonic Callus, etc. among those friable callus (loosely packed callus) is preferred. If friable callus is not producing it can be induced by certain treatments. When the friable callus is put in liquid medium and kept in shaker the cells of the callus will get dissociated. Usually 75ml of the media is taken in 250 ml flask, the optimum inoculating density is 5 mg/75ml, and the pH of the medium should be 5.6 the flask is kept in a Gyratory Shaker at 120 rpm, which is for the aeration of the medium.

Sub Culturing

Sub culturing can be done when the cells are in the exponential stage, the stage of development can be identified by growth pattern studies. The culturing can be done by batch culture or by continuous culture. One of the method is 5 ml of inoculum from the suspension [75 ml] is transferred 70 ml of fresh medium. Like this way 15 or more flasks of culture can be produced. Another method is that, divide the media equally into two parts and add the fresh media to make-up to 75 ml and this process is continued.

For identification of the stage and analysis of the product growth pattern studies are very much important. Growth study is done for 25 days form the starting. For studying growth different methods (Optical Density, Dry weight, Cell counting) can be used. By this we can know the Packed Cell Volume (PCV) along with that content analysis also can be done so we will get a clear idea that on particular day at a particular stage of growth the cell is producing the secondary metabolite. Moreover the target of the product also can be known, ie. Whether it is produced Intracellular or Extracellular.

Selection of cell lines for high yield of secondary metabolites

The purpose of tissue culture is to produce high amount of secondary metabolite, but in general majority of the callus and suspension cultures produce less quantity of secondary metabolites. The reason for this is mainly due to the lack of fully differentiated cells in the cultures. There are some techniques ultimately useful for the separation of producers and non-producers. The techniques employed for this are cell cloning, visual and chemical analysis and selection for resistance.

Large Scale Cultivation of Plant Cells

To achieve industrial production of the desired metabolite, large-scale cultivation of the plant cell is required. Plant cells when cultured exhibit changes in volumes and thus variable shapes and sizes. Further, cultured cells have low growth rate and genetic instability. All these aspects have to be considered for the mass cultivation of cells.

The following four different culture systems are widely used

1. The Free-Cell Suspension Culture
2. Immobilized Cell culture
3. Two Phase System culture
4. Hairy root culture

The Free-Cell Suspension Culture

Mass cultivation of plant cells is most frequently carried out by cell suspension cultures. Care should be taken to achieve good growth rate of cells and efficient formation of the desired secondary metabolite. Many specially designed bioreactors are in use for free-cell suspension cultures.

Bioreactors for the Production of Secondary metabolites

- Batch Bioreactors
- Continuous Bioreactors
- Multistage Bioreactors
- Airlift Bioreactors
- Stirred Tank Bioreactors

Two important aspects should be considered for good success of suspension cultures

- Adequate and Continuous Oxygen Supply
- Minimal generation of hydrodynamic stresses due to aeration and agitation.

Immobilized Cell Cultures

It is possible to render plant cells immobile or immovable for use in culture systems. Entrapment causes the cells to become physically immobile. In addition to individual cells, aggregate cells and even calluses can be immobilized. For immobilization, uniform cell suspensions are better.

Surface immobilized plant cell (SIPC)

Technique effectively maintains the cells and promotes faster cell growth. Better cell-cell interaction and protection from severe shear and pressures are both achieved when cells are immobilized. These all contribute to the highest possible generation of secondary metabolites.

The common methods used for entrapment are.

Entrapment of cells in gels: Several gels can immobilize the cells or protoplast. For instance: Alginate, Agarose, Carrageenan You can use the gels separately or in combination. The methods used to immobilize plant cells are similar to those employed to immobilize microorganisms or other types of cells.

Entrapment of cells in nets or foams: Several gels can immobilize the cells or protoplast. For instance: Alginate, Agarose, Carrageenan You can use the gels separately or in combination. The methods used to immobilize plant cells are similar to those employed to immobilize microorganisms or other types of cells.

Entrapment of cells in hollow-fibre membranes: For cell immobilization, cellulose acetate silicone polycarbonate tubular hollow fibers arranged into parallel bundles are utilized. Between the fibers, cells may become trapped.

Bioreactors for use of immobilized cells
Immobilized cells are used for large-scale culturing in fluidized bed or fixed bed bioreactors. In fluidized bed bioreactors, the medium is pumped or an air flow is used to stir the immobilized cells. In contrast, immobilized cells were maintained stationary (not stirred) and slowly perfused with an aerated culture medium in the fixed bed bioreactor. the list of plant species whose cells were immobilized and used to produce secondary metabolites.

Two phase system culture
Immobilized cells are used for large-scale culturing in fluidized bed or fixed bed bioreactors. In fluidized bed bioreactors, the medium is pumped or an air flow is used to stir the immobilized cells. In contrast, immobilized cells were maintained stationary (not stirred) and slowly perfused with an aerated culture medium in the fixed bed bioreactor. the list of plant species whose cells were immobilized and used to produce secondary metabolites.

Hairy root culture
Hairy root cultures are for the production of root-associated metabolites. In general, these cultures have high growth rate and genetic stability.
For the production of hairy root cultures, the explant material is inoculated with pathogenic bacterium, *Agrobacterium rhizogenes.* The organism contains root-inducing (Ri) plasmid that causes genetic transformation of plant tissues, which finally results in the hairy root cultures. Hairy roots produced by plant tissues have metabolite features similar to that of normal tissues.
The production of secondary metabolites in plant cultures is generally low and does not meet the commercial demands. The synthesis of majority of secondary metabolites involves multistep reactions and many enzymes. It is possible to stimulate any step to increase product formation.
Elicitors are the compounds of biological Orgin, which stimulate the production of secondary metabolites, and the phenomenon of such stimulations are called as elicitation. Elicitors produced within the plant cells are called as endogenous elicitors (Eg: Pectin, pectic acid and other polysaccharides) when the elicitors are produced by microorganisms they are called as exogenous elicitors (Eg: Chitins, Glucans). All the elicitors of biological orgin are biotic elicitors. Physical (Cold, Heat, UV light, etc.) and chemical agents (ethylene, fungicides, antibiotics) can also increase product formation such elicitors are called as abiotic elicitors.

Factors affecting secondary metabolite production
There are various factors affecting Secondary metabolite production, some of the major factors includes:
- Light
- Incubation Temperature
- pH of the medium
- Aeration of the culture, etc.

All these factors will have determine effect on the production of secondary metabolite.

Production of secondary metabolites from medicinal plants by plant tissue cultures
The following are the main benefits of a cell culture system over the traditional growing of full plants:
- Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions.
- Cultured cells would be free of microbes and insects.
- The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites.
- Automated control of cell growth and rational regulation of metabolite processes would reduce labor costs and improve productivity.

As they are utilized as protections against predators, parasites, and diseases, for interspecies competition, and to help the reproductive processes (coloring agents, alluring scents, etc.), these compounds typically have an ecological purpose or are important to the organism.

Classified as
- Alkaloids
- Terpenoids
- Phenolics

- Alkaloids are substances that come from plants, are basic, include one or more nitrogen atoms (often in a heterocyclic ring), and typically have a pronounced physiological effect on people or other animals.
- Terpenes are a diverse and expansive class of hydrocarbons that are mostly produced by a wide range of plants, especially conifers. Terpenoids are the name given to the chemicals created when terpenes undergo chemical modification, such as oxidation or rearranging the carbon skeleton. Isoprenoids and terpenoids are synonyms.
- Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Terpenes are derived biosynthetically from units of isoprene, which has the molecular formula C_5H_8.
- Phenols, often known as phenolics, are a category of chemical compounds-made composed of an aromatic hydrocarbon group and an attached hydroxylgroup (-OH). Phenol (C6H5OH) is the most basic member of the group. The precursors of phenols are
  - Tannins
  - Chalcones
  - Lignans
  - Flavonoids - flavonoids, isoflavonoids, neoflavanoids.
  - Anthocyanins
  - Coumarins

Aloe vera
Family *Liliaceae*

Fig 1: Plant Stature of *Aloe vera*

- It is a stemless (60–100 cm) tall, spreading by offsets.
- The leaves are lanceolate, thick and fleshy, green.
- The margin of the leaf is serrated and has small white teeth.
- The flowers are produced in summer on a spike.
- *A. vera* forms Arbuscular mycorrhiza, succulent plant.

Leaves contain a range of biologically active
- Glycoside ALOIN - 3%
- Acetylated Mannans
- Pollyanna's
- C-glycosides,
- Anthraquinones
- Water soluble glycoside Barbaloin-3.8%

Biologically active compounds

Aloein

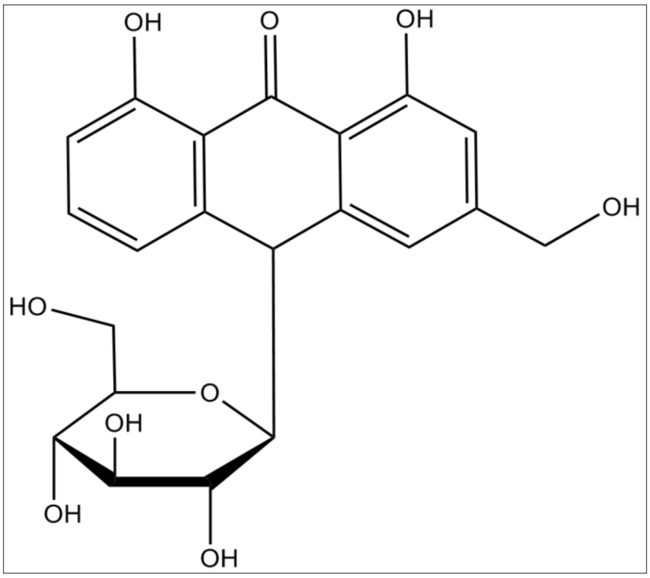


Fig 2: Composition of Aloein

Uses
- *Aloe vera* has a long association with herbal medicine.
- *Aloe vera* is alleged to be effective in treatment of wounds.
- Anti-inflammatory
- Immunostimulant
- The consumption of *A. vera* has been associated with reduced blood sugar levels in diabetics.
- Preliminary research indicates that oral *A. vera* gel may help ulcerative colitis patients experience less symptoms and less inflammation.
- Effective against lesions.

Andrographis paniculata
Family *Acanthaceae*

Fig 3: Plant stature of *Andrographis paniculata*

- It has glabrous leaves and grows erect to a height of 30-110 cm in damp, shady areas.
- White flowers with petals spotted with rose-purple.
- Dark green stem; lanceolate, pinnate leaves up to 8.0 cm long and 2.5 cm wide; glabrous.
- Small flowers, many sub-quadrate, yellowish-brown seeds.

Biologically active compounds

Chief constituent extracted from the leaves of the plant
- Andrographolide - 2.5%
- Andrographine
- Neoandrographolide
- Panicoline
- Paniculide-A
- Paniculide-B
- Paniculide-C

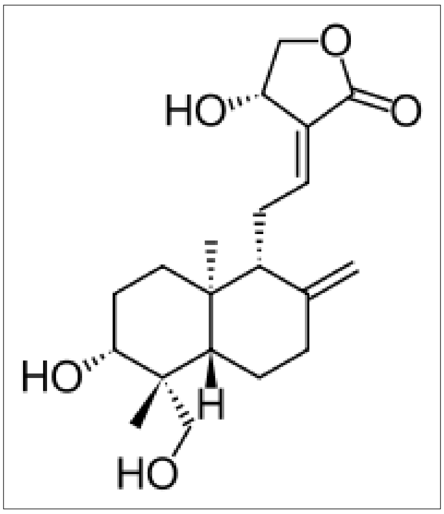


Fig 4: Composition of *Andrographis paniculata*

Uses
- Antityphoid
- Antifungal activities
- Antibiotic
- Antimalarial
- Antihepatitic
- Antithrombogenic
- Antiinflammatory
- Antisnakevenom
- Antipyretic
- Immunostimulant agent
- A recent study conducted at Bastyr University, confirms anti-HIV activity of andrographolide.
- Hepatoprotective
- Antihypoglycaemic
- Febrifuge
- Cholagogue action
- Decoction – Blood purifier

Withania somnifera
Family *Solanaceae*

Fig 5: Plant stature *Withania somnifera*

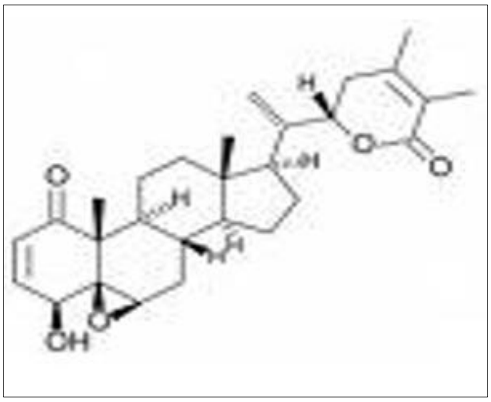
- It develops into a robust shrub with 170 cm of yellow blooms.
- Red fruit and fruit is berry.

Biologically active compounds

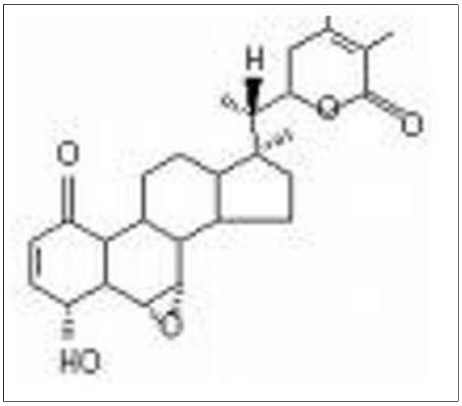
The root is the part used – 0.13% Alkaloids

- Withananine (alkaloid)
- Withanolides A-Y(steroidal lactones)
- Anaferine (alkaloid)
- Anahygrine (alkaloid)
- Chlorogenic acid (in leaf only)
- Cuscohygrine (alkaloid)
- Pseudotropine (alkaloid)
- Scopoletin
- *Somniferum*
- Withaferin A (steroidal lactone)
- Withanine (alkaloid)
- Coumarins – Scopoletin & Aesculetin

(The withanolides have C28 steroidal nucleus with C9 side chain, having six membered lactone ring.)



With an iferine A



With a none

Fig 6: Composition of *Withania somnifera*

USES

- Ashwagandha is categorized as a rasayana herb in Ayurveda, which promotes general health and longevity.
- Stimulates neuroendocrine system.
- The plant's berries and roots are used to make herbal remedies.
- *Withania somnifera* is an Indian medicinal plant whose fruits, leaves, and seeds have long been utilized in Ayurvedic medicine.
- Aphrodisiacs,
- Diuretics
- For treating memory loss.
- For promoting reproductive fertility.
- Alleviate symptoms associated with arthritis.
- The substance known as "ashwagandha oil" is a blend of ashwagandha, almond oil, and rose water intended to be used as a skin toner, thus it shouldn't be swallowed.
- Nuclear factor-kappaB expression is reduced, which has anti-carcinogenic effects in animal and cell cultures.
- Intercellular tumor necrosis factor is suppressed, and apoptotic signaling is amplified in malignant cell lines.

Tulsi

Ocimum Sanctum

Family *Lamiaceae*.

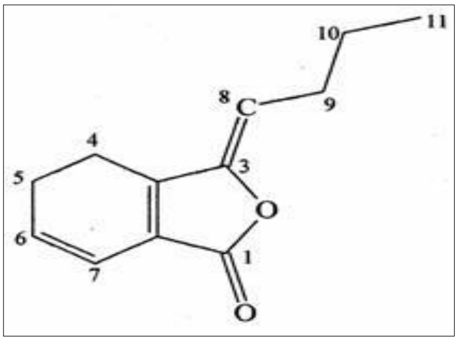
Fig 7: Plant stature of Tulsi

- It is an erect, much branched subshrub 30-60cm tall.
- Leaves simple opposite green or purple & are strongly scented and hairy stems.
- Flowers are purplish in elongate racemes in close whorls.

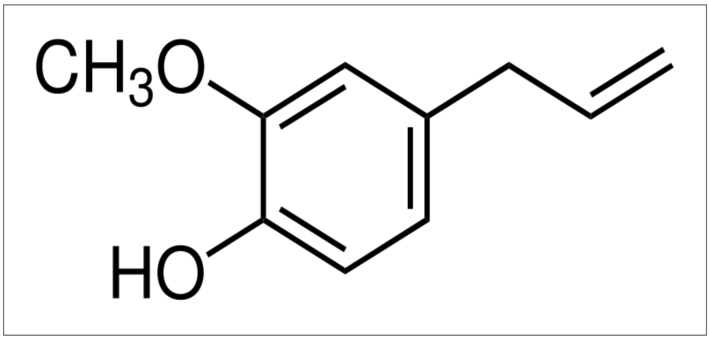
Biologically active compounds

Leaves, seeds, stem

- Ursolic acid
- Rosmarinic acid
- Eugenol - 0.0075%-0.095%
- Carvacrol
- Linalool
- β-caryophyllene
- Thymol
- Humulene



β-caryophyllene



Eugenol

Uses

Tulsi's extracts are used in ayurvedic remedies for

- Common colds
- Headaches
- Stomach disorders
- Inflammation
- Heart disease
- Various forms of poisoning and malaria.
- Essential oil extracted from Karpoora Tulsi is mostly used for medicinal purposes.
- Used in skin preparations due to its anti-bacterial.
- Recent research suggests that Tulsi's high eugenol (1-hydroxy-2-methoxy-4-allylbenzene) concentration may make it a COX-2 inhibitor, like many contemporary analgesics.
- Diabetes is effectively treated by lowering total cholesterol and blood glucose levels.
- Antioxidant
- Protection from radiation poisoningand cataracts.

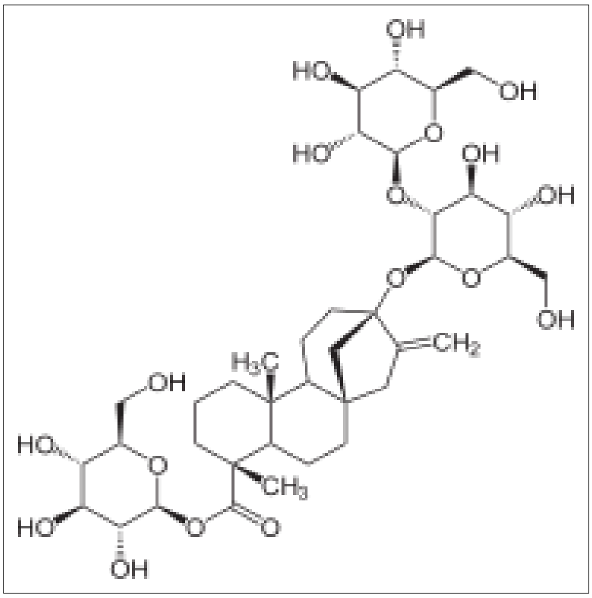
Stevia rebaudiana
Family *Asteraceae*

Fig 7: Plant stature of Stevia

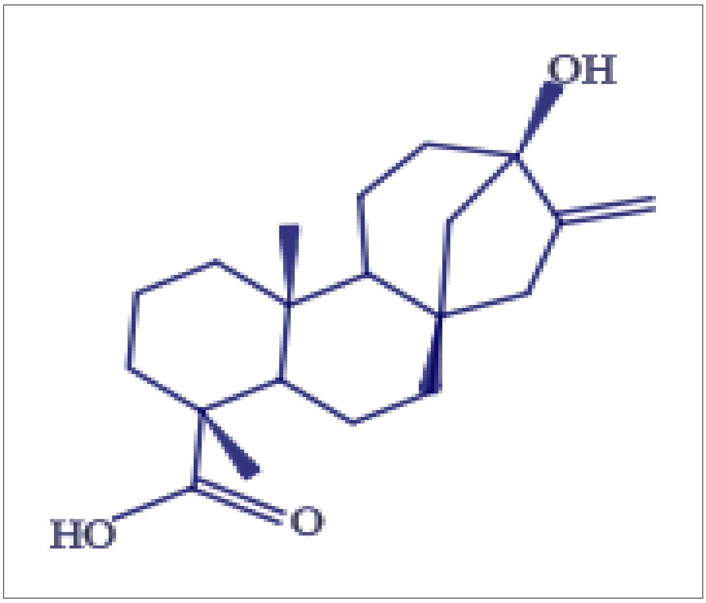
- *Stevia* is genus of herbs and shrubs.
- Commonly known *as sweet leaf or sugar leaf.*
- Flowers are white.

- Steviol glycosides in the stevia leaf.
- Rebaudioside 2–4% rebaudioside A — most sweet (350–450X of sugar) and least bitter.
- Stevioside 5–10% stevioside (250–300X of sugar).

Biologically active compounds



Stevioside



Rebaudioside

Uses

- As a sugar substitute for, stevia offers a longer-lasting and slower-onset flavor than sugar (with extracts up to 300 times sweeter than sugar).
- Stevioside has demonstrated some pharmacological effects in people with type-2 diabetes and hypertension.
- Low-sugar food alternatives. low-carbohydrate.
- Treating conditions such as obesity and high blood pressure.
- Stevia has no discernible impact on blood glucose levels, even though it increases glucose tolerance.
- Given on carbohydrate-controlled diets.

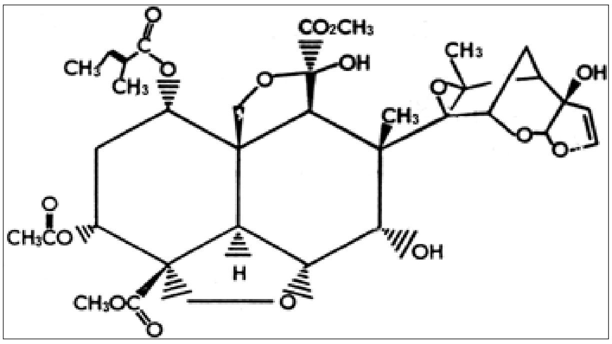
Neem
Azadiracta indica
Family *Meliaceae*

Fig 8: Plant stature of Neem

- Neem trees grow quickly and can go as tall as 15 to 20 meters.
- Despite being evergreen, it may lose most or almost all of its leaves after a severe drought.
- The branches are dispersed widely. The trunk is quite small, straight, and can grow up to 1.2 meters (approximately 4 feet) in diameter. The bark is whitish-grey to reddish-brown, rough, fissured, or scaly.

Biologically active compounds
The seeds, leaves, blossoms, and bark of the tree are all utilized to make a variety of various medicinal remedies. Azadiractin - 3%

- Nimbin
- Nimbidine
- Limonine
- Vepinine
- Salanin
- Vilasinin
- Margosene Flowers



Nimbi dine

USES
In India, the tree is variously known as "Divine Tree," "Heal all," "Nature's Drugstore," "Village Pharmacy" and “Panacea for all diseases.”

- Anthelmintic
- Antifungal
- Antidiabetic
- Antibacterial
- Antiviral
- Anti-fertility
- Sedative
- Emollient
- Astringent
- Diuretic
- Tonic
- Used against leprosy, sprain, rheumatism, piles.
- Traditional Indian medical practitioners advise patients with chicken pox to lie on neem leaves.

***Catharanthus roseus* (Madagascar Periwinkle)**
Family *Apocynaceae*

Fig 9: Plant stature of Madagascar Periwinkle

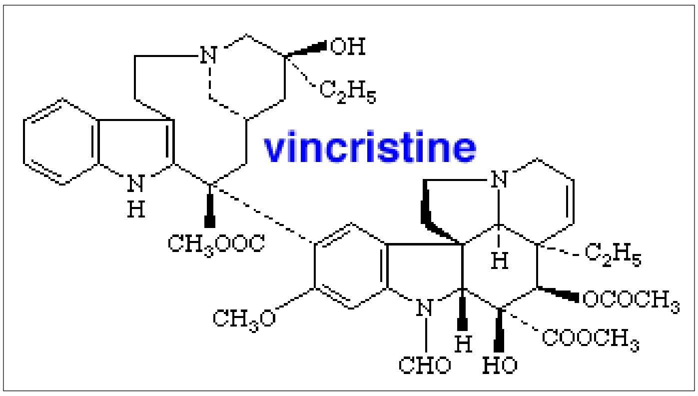
- It is an evergreen subshrub or herbaceous plant growing to 1 m tall.
- The leaves are oval to oblong.
- The blooms have a basal tube that is 2.5–3 cm long, a corolla, and are white to dark pink with a darker red center.
- Two follicles make up the fruit.

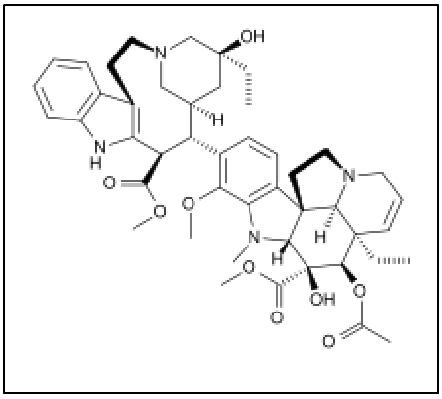
Biologically active compounds

- Roots, leaves, flowers, seeds
- Total Alkaloid content

In roots-1.34%
In leaves-1.16%
In seeds 0.18

- Vincristine
- Vinblastine
- Reserpine
- Ibogaine
- Vindoline
- Leurosine
- Lochnerine
- Tetrahydroalstonine
- Ursolic acid





Vinblastine

USES

Herbal medicine used to treat numerous diseases including

- Malaria and Diabetes.
- Vinblastine and vincristine, which are made from the plant, are used to treat leukemia.
- Vincristine is exclusively administered intravenously and utilized in a variety of treatment regimens.
- Its primary applications are in the treatment of acute lymphoblastic leukemia and non-Hodgkin's lymphoma as part of chemotherapy.
- Nephroblastoma, a kidney tumor commonly found in youngsters that is being treated.
- In rare cases, it is used to suppress the immune system, such as in thrombotic thrombocytopenic purpura (TTP).

Conclusion

- In future, metabolic engineering and biotechnological approaches can be used as an alternative production system to overcome the limited availability of biologically active, commercially valuable, and medicinally important plant secondary metabolite compounds.
- Advances in biotechniques, particularly methods for culturing plant cell cultures, should provide new means for the commercial processing of even rare plants and the chemicals they provide.
- The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products. The major advantage of the cell cultures includes synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions.
- The use of *in vitro* plant cell culture for the production of chemicals and pharmaceuticals has made great strides building on advances in plant science. The increased use of genetic tools and an emerging picture of the structure and regulation of pathways for secondary metabolism will provide the basis for the production of commercially acceptable levels of product.
- Knowledge of biosynthetic pathways of desired phytochemicals in plants and in cultures is often still in its infancy, and consequently strategies needed to develop an information based on a cellular and molecular level. These results show that *in vitro* plant cell cultures have potential for commercial production of secondary metabolites.
- The introduction of newer techniques of molecular biology, so as to produce transgenic cultures and to effect the expression and regulation of biosynthetic pathways, is also likely to be a significant step toward making cell cultures more generally applicable to the commercial production of secondary metabolites.
- The commercial values of plant secondary metabolites have been the main impetus for the enormous research effort put into understanding and manipulating their biosynthesis using various chemical, physiological, and biotechnological pathways.
- *In vitro* propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds.
- The increased use of plant cell culture systems in recent years is perhaps due to an improved understanding of the secondary metabolite pathway in economically important plants. Advances in plant cell cultures could provide new means for the cost-effective, commercial production of even rare or exotic plants, their cells, and the chemicals that they will produce.
- Knowledge of the biosynthetic pathways of desired compounds in plants as well as of cultures is often still rudimentary, and strategies are consequently needed to develop information based on a cellular and molecular level.
- A key to the evaluation of strategies to improve productivity is the realization that all the problems must be seen in a holistic context. At any rate, substantial progress in improving secondary metabolite production from plant cell cultures has been made within last few years.
- These new technologies will serve to extend and enhance the continued usefulness of higher plants as renewable sources of chemicals, especially medicinal compounds.
- We anticipate that continued and increased efforts in this area will result in the effective biotechnological synthesis of particular, valuable, and as-yet-unknown plant compounds.

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