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PLANT AND CROP PHYSIOLOGY – PRINCIPLES AND MODERN CONCEPTS

Review Based Book Chapter

PLANT TISSUE AND CELL CULTURE: A POWERFUL TOOL FOR ENHANCED PRODUCTION OF TARGETED ACTIVE COMPOUNDS IN MEDICINAL PLANTS

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REVIEW BASED BOOK CHAPTER

PLANT TISSUE AND CELL CULTURE: A POWERFUL TOOL FOR ENHANCED PRODUCTION OF TARGETED ACTIVE COMPOUNDS IN MEDICINAL PLANTS

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Abstract

Medicinal plants are core therapeutic essentials in various human health problems. Interest in the usage of plants and plant-based products has resurfaced around the globe as more people become conscious of the health threats with the indiscriminate use of antibiotics. Tissue culturing enables the preservation of most of the medicinal plant's genetic material which is at the risk of getting endangered. During the last couple of years, the emerging pharmacological significance of secondary metabolites has led to a high interest in secondary metabolism, predominantly in the potential for the development, through tissue culture technologies, of bioactive plant metabolites. This study compiles the methods used for the synthesis of secondary metabolites like taxols, anthocyanin, colchicine, ginkgolides, L-Dopa, and diosgenin from various medicinal plants using plant tissue culture techniques are discussed briefly. Advances in the plant tissue culture techniques and media formulations that are essential for the progression of medicinal plants and their useful metabolites have also been reviewed.

Keywords
Medicinal Plants, Secondary Metabolites, Tissue Culturing Techniques, Taxols, L-DOPA, Diosgenin, Colchicine, Anthocyanin, Gankgolides

1. Introduction

Plants produce a lot of metabolites (secondary) that are highly beneficial for survival of plants in the natural environment. Secondary products of plants are those compounds that have no role in the maintenance of biological processes in plants. These metabolites are directly linked with the interlinkage of plants with their natural environment in which they grow [1].

Naturally, when plants grow, a lot of factors like the location of the plant inland and drastic situations of environment can limit their growth. To avoid these restrictions in the growth of beneficial plants, the tissue culturing technique is used nowadays [2]. These secondary products in plants act as defensive material against the attack of pathogens and as an attractant to proceed the process of pollination for reproduction.

Scientists are highly intrigued to synthesize and enhance secondary metabolites production on commercial scale because of their increasing use as pharmaceuticals and neutraceuticals. For this purpose, tissue culturing has been found highly valuable in the research area [3, 4]. Tissue culturing of therapeutic plants plays very important role in secondary metabolite production at an industrial level that is pharmaceutically very beneficial in the treatment of different diseases. The genetic material of different highly threatened plants can also be conserved by applying tissue culturing techniques to them [5].

Plant tissue culturing means the in vitro propagation of a complete plant from any small part of the plant like a small cell, organ, or tissue in an aseptic environment [6]. A lot of techniques are used for the culturing of medicinal plants like callus cultures, suspension cultures, micro-propagation and protoplast cultures, etc. Culturing medicinal plants can be done in different mediums like liquids, solids, etc. But, most of the secondary products are synthesized by culturing plants in a liquid medium like cell suspension culture because in the liquid medium uniform amount of nutrients are available to the plant allowing uniform and rapid growth of the medicinal plant so that a large number of secondary metabolites can be extracted from them [7].

This review summarizes the information of useful techniques of tissue culturing that play an important role in developing a complete medicinal plant as well as the six most important secondary metabolites extracted from the medicinal plants through tissue culture is also being discussed to highlight their pharmacological application.

2. Tissue Culture Techniques

There are many tissue culture techniques used nowadays. Some of them used for culturing medicinal plants are discussed below.

2.1. Callus Cultures

When ex-plants are grown on nutrient medium an undifferentiated tissue mass is visible with in some days that is known as callus [8]. Growth hormones play a significant role in this tissue culture technique. Dedifferentiation and re-differentiation both processes can cause callus formation in ex-plants [9]. For example, *Cardiospermum halicacabum* Linn. is an important Indian medicinal herb. Its seeds are used to cure secondary hyperhidrosis whereas, its roots and leaves are helpful in treating fever, arthritis etc. It imparts tranquillizing effect on central nervous system. The extracts of this plant have shown antifilarial activity [10].

The calli could be developed by using Murashige and Skoog (MS) medium on which the nodal and leaf segments were cultured by T. Dennis Thomas et al [11]. Various chemical supplements can be added to induce callus formation such as 2,4-dichlorophenoxy acetic acid. Increase in shoot regeneration was reported when different concentration of kinetin and 6-benzyladenine was used with or without mixing of indole 3- acetic acid in development media [11].

2.2. Suspension Culture

In suspension cultures solitary cells or petite cell groups such as callus are grown on liquid culture medium and constant agitation is provided that helps to break large cell masses [8, 9]. Suspension culture is in vitro technique and it requires continuous gas exchange, normally conical flasks are used as it allows gaseous exchange due to its large surface area [9]. The advantage of this technique is that extraction is much easier, cell grows at faster rate and product yield is higher than field grown plants [12]. Two types of culture techniques are mostly used. Continuous cultures open cultivation system is used whereas, fresh nutrients are added and waste is extracted at regular intervals [9]. Benefit of continuous culture is the product yield is higher but it enhances the chance of contamination. The second type is batch cultures that is closed system and no fresh media or cell suspension is added or removed during the process as it is taken out or added after a certain period of time and it minimizes the chance of contamination [8].

Secondary metabolites are widely produced on industrial scale by using suspension cultures [13]. Many natural products had been produced for example: resveratrol [14, 15], ajmalicine [16], paclitaxel [17], ginsenosides [18], and artemisinin [19].

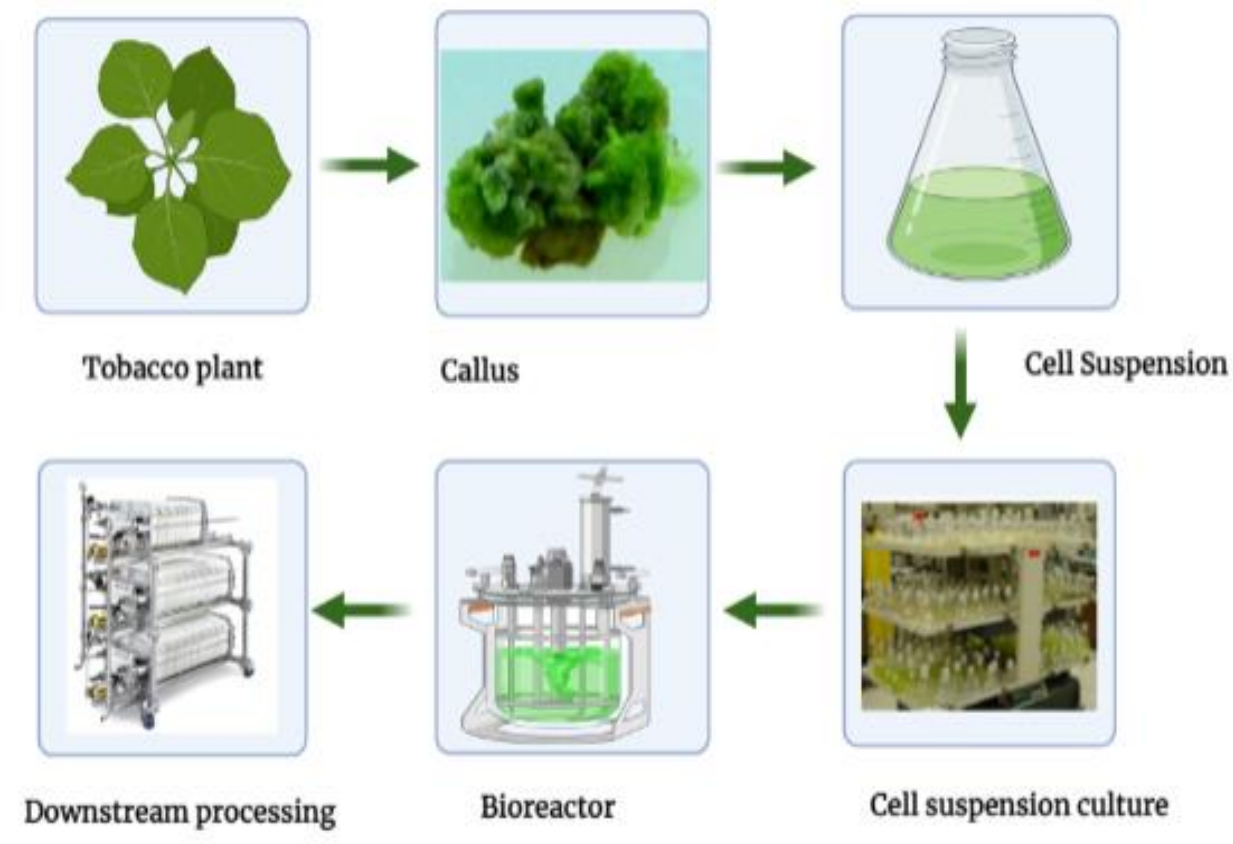


Figure 1. Suspension culture of Tobacco plant

A study had been carried on suspension cultures of *Gentiana davidii* in China. 400 species of genus *Gentiana (Gentianaceae)* is distributed worldwide [20]. In Taiwan, *Gentiana davidii var. Formosana* is most commonly found [21]. Many Gentiana species are used to prepare traditional Chinese medicines and bitter tonics that is due to its bitter principles [22]. In suspension cultures of *Gentiana davidii var. Formosana* cells shaker speeds, incubation period, plant growth regulators (auxins), and pH, etc can affect cell growth [23]. Stem-derived callus was used and grown on MS basal medium having varying concentration of kinetin, 2,4-D, NAA, IAA and sucrose. It was observed that 0.2mg kinetin and sucrose (3%) in medium, pH between 4.2-5.2, and shaker speed at 60-120rpm helps in optimal cell growth [24].

Table 1. Products produced by medicinal plants and their use

Product	Plant species	Use	Reference
Saponin	*Panax notoginseng*	Efficient tumor promoter inhibitor	[25]
Resveratrol	*Vitis vinifera*	Pharmacological and biological actions	[26]
Anthocyanin	*Perilla frutescens*	Anti-diabetic, anti-cancer, anti-obesity effects	[27]
Ajmalicine	*Catharanthus roseus*	Anti-hypertensive drug	[28]
Crocin	*Crocus sativus*	Painkiller	[29]
Taxane	*Taxus media*	Used in cancer treatment	[30]
Paclitaxel	*Taxus cuspidate*	To treat breast and ovarian cancer	[17]

2.3. Somatic Embryogenesis

Somatic embryogenesis is the formation of non-zygotic embryo i.e. an embryo derived from a cell or tissue instead of gametes fusion. Generally, an auxin rich medium is used where callus is grown that forms embryonic clumps which are afterwards grown on other medium (without auxin) to form mature embryos whose formation and growth both depends upon nitrogen and auxin concentration in medium [8, 31].

Somatic embryogenesis has made its way to recognization by providing fast propagation and preserving Date palm *(Phoenix dactylifera L.)*. Grown in Middle East and North Africa, that plant is known from centuries for its use as human food and its benefits to human body. *Phoenix dactylifera* phenolics study represents its involvement in activities such as;

- Anti-aging, antioxidants, anti-cancerous, antimicrobial and antiviral properties to cure acute or chronic inflammations and diseases [32]
- Anti-thrombotic effects are induced by phenols present in date palm that helps to reduce blood pressure [33, 34]
- Lymphocytes during their phagocytosis activity causes oxidative damages that can be recovered by phenols [35]

Phoenix dactylifera forms approximately 20 offshoots in initial 10-15 years that limits its availability. Somatic embryogensis is effective method for commercial scale production of date palm and provides future goals for plant breeding programs [36]. Various supplements can be added into the medium to enhance the embryo growth and formation such as abscisic acid, biotin [37] and coconut water additive improves somatic embryogenesis [38, 39].

2.4. Protoplast Cultures

Protoplasts are formed when either chemical or mechanical means are used to degrade the plant cell wall. A solution having high concentration of solute (hypertonic) was conventially used previously for protoplasm formation that causes the plant cell wall to shrink. Now a days cellulases and pectinases are used for enzymatic digestion of cell wall. *A. judaica and E. spinosissimus* had been regenerated by using protoplast culture.

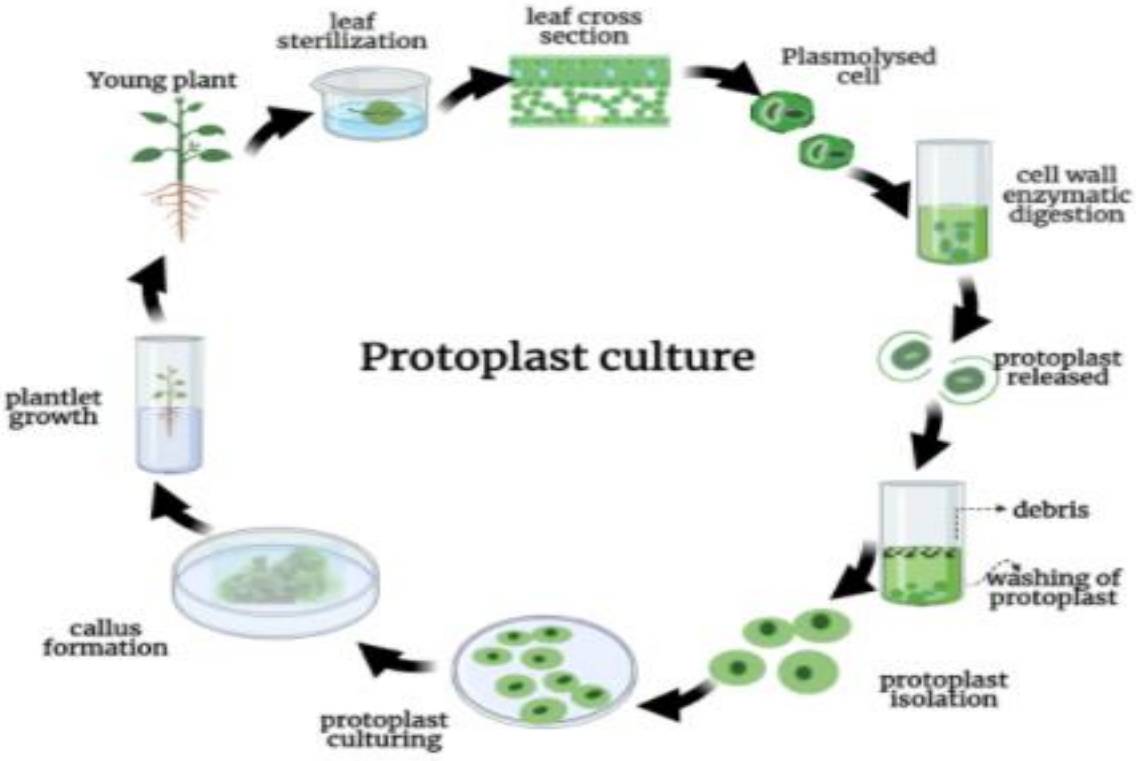


Figure 2. Protoplast culture

3. Plant Tissue Culture for Production of Secondary Metabolites from Medicinal Plant

Secondary metabolites of plants are of great usage for mankind. Plants are the main source of secondary metabolites. Plants secondary metabolites are used for purpose of flavoring, medicines and pigments. Six secondary metabolites: taxols, anthocyanin, colchicines, ginkgolides, L-DOPA and diosgenin are discussed below along with their medicinal uses and production by tissue culturing of medicinal plants.

3.1. Taxols

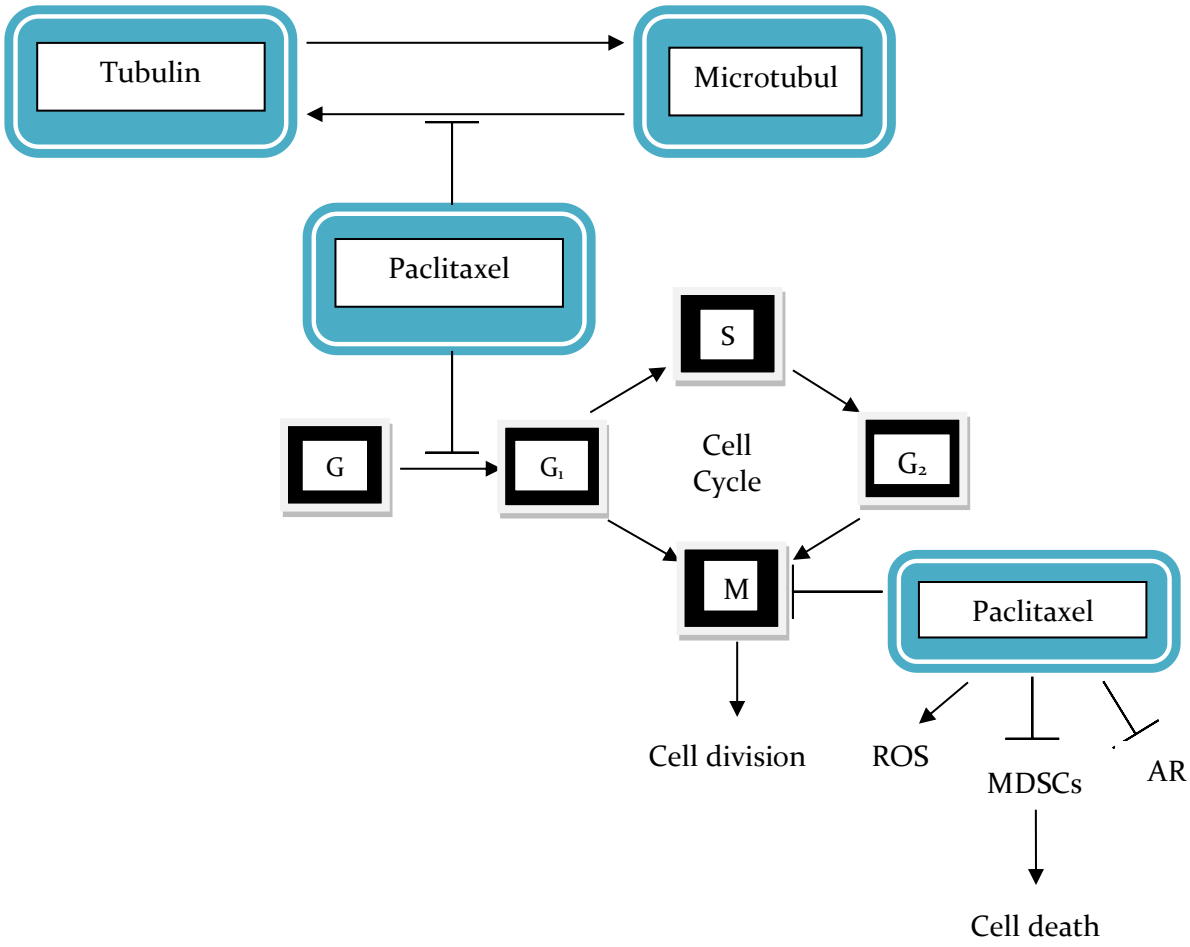
Taxol is one of most important diterpenes. It stabilizes the microtubule and act as mitosis inhibitor in cancerous cells. In cancer cells, it also alternates the biochemical pathways and interfere with protein for cancerous cell death. Baccatin III is common in all taxanes, which are composed of oxetan ring (4 membered). They contain ester linkage at C-13 that is responsible for its antitumor property. Interaction of tubulin with taxols results in microtubule stabilization and produces cytotoxicity [40]. Cancerous cell division is inhibited by taxol when they bind to the surface of microtubules specially to tubulin heterodimers subunit. This enhances polymerization of microtubules even in GTP absence [41]. Taxols are mainly used for treating ovarian metastatic carcinoma [42], and other cancerous types: lung, breast, prostate, colon, and brain [43].

The molecular formula of taxol is C47H51NO14 with mol wt. 853.9 Da [44]. Its core structure contains ring systems. These rings consist of some important groups: 2 hydroxyl, 2 acetyl, 1 benzoyl group and 1 oxetane ring. A side chain is bound with carbon number 13 of core with two functional groups: benzoyl and OH groups.

Different plants yield varying concentration of taxol and its derivatives, along with principal components like baccatin III, docetaxel & 10-deacetyl baccatin to be greater in extracts of leaf as compared to bark extracts [45]. The carbohydrate addition throughout growth cycle enhances taxol production rate [46]. Taxus species are not only the source of taxol but also yields other taxoids [47]. When amino acids (more effective is phenylalanine) are added to Taxus cuspidata culture medium, it enhances production of taxoids [48]. Nguyen et al. studied factors that control the taxol stability

and recovery from suspension culture [49]. Linden et al. stated effects of concentration of gas and rare earth elements on manufacturing of taxol [50, 51].

Figure 3. Anticancerous action of Taxol



Cusidó et al. have shown in their study that fosmidomycin and mevinolin addition blocks the synthesis of taxol in cell cultures of *Taxus baccata* [52]. For initiating callus formation young tissues are reported to be extra responsive as compared to mature plant parts [53-55]. The best strategy for inducing callus is using kinetin in combination with 2,4-D [56]. Bruňáková et al. reported an enhanced callus growth from taxus specie when implanted in B5 medium [53]. When callus becomes old it produces high concentration of taxols [53-55, 57]. For growth of cells, B5 media was used containing 0.5% fructose, 0.1 mg/L 6-benzylaminopurine, 0.5% sucrose & 2.0mg/L 1-naphthaleneacetic acid. Then for paclitaxel production, the cells were shifted to

another media supplemented with 3% sucrose, 0.1mg/L kinetin and 2.0mg/L picloram (2.0 mg/L) (58-60]. Taxol production is enhanced when media is enriched by fructose while addition of glucose inhibits its production. When *Taxus canadensis* and *Taxus cuspidata* cell culture is supplemented with Methyl jasmonate production of paclitaxel is enhanced. The addition of cobalt chloride, ammonium citrate, silver nitrate, salicylic acid and vanadyl sulphate causes increased production of taxane in cell cultures of *T. baccata* [61, 62] .

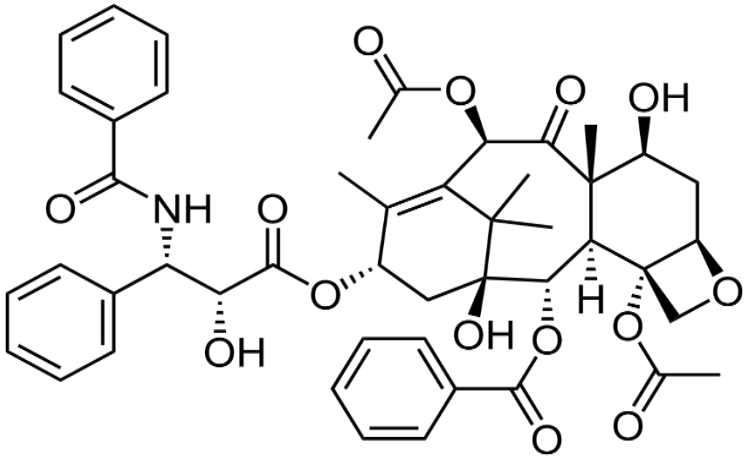


Figure 4. Structure of Taxol [62]

3.2. Anthocyanins

Anthocyanins are extensively found pigments in kingdom of plants. These pigments give red-blue color to plants [63]. Anthocyaninis have antioxidant property and thus they are considered to be beneficial component of diet [64]. They are anti-cancerous components and have healthy effects against diabetes, neurological and cardiovascular diseases [65].

Anthocyanins belong to the class of flavonoids. They contain anthocyanidin which helps in its structural stabilization. The sugars present in anthocyanins are rhamnose, galactose, xylose, fructose, glucose and arabinose [66]. Anthocyanin varies in color in different ranges of pH. Anthocyanins act to function as UV filter present on leaves [67]. It also provides pathogenic resistance in some herbivores [68] and plant species [69, 70].

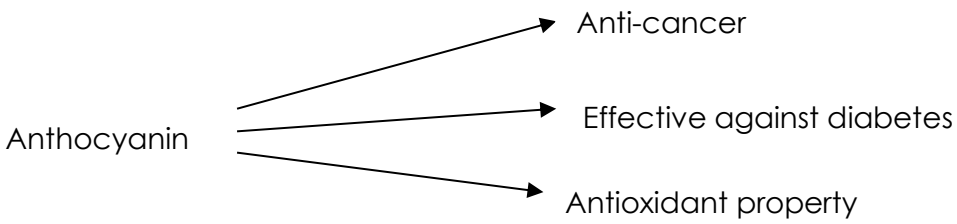


Figure 5. Medicinal importance of Anthocyanin

Anthocyanins are extracted as by product in production of wine from grapes seeds and skin. As grapes are seasonal fruit so the production is limited [71]. Many plant species are used for anthocyanin production by cell and tissue culture techniques including Vitis, Vaccinium pahala, wild carrot, Ajuja reptans and strawberry species. Induction of reddish pink colored spots in plant callus culture is indication of production of anthocyanins. Generation of pigment is enhanced by mechanical isolation and sub-culturing of cell repeatedly [66].

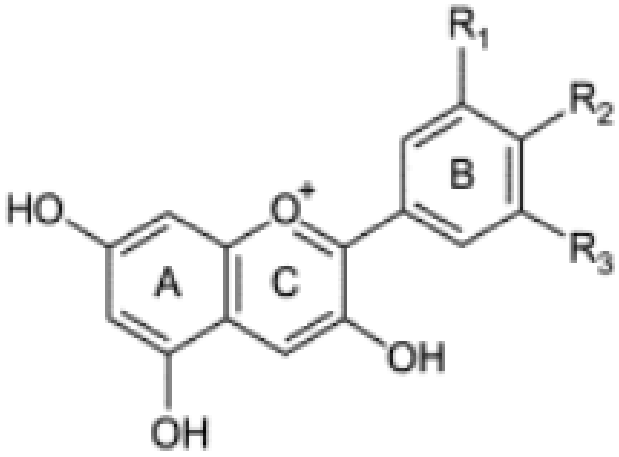


Figure 6. Structure of Anthocyanin [72]

Dimorphotheca sinuata is branching and an annual plant that contain obtuse and narrow based leaves. Ernest A. et al. used the stem of *Dimorphotheca sinuate* for production of anthocyanin. They cut the stems and dried in oven, placed in Murashige and Skoog agar medium containing 3% sucrose. They also studied the influence of

auxins on anthocyanin production. For this purpose, different categories of auxins were inserted into callus culture. After one month, callus growths were subcultured. They observed that the culture media containing OCPAA have produced anthocyanin in greater concentration. They separated 10g of callus tissue and extracted pigment by using CH_3OH following its precipitation. Chromatographic techniques were used for separating anthocyanins followed by their identification with spectro-photometry. They studied two anthocyanins (delphinidin-3-glucoside and cyanidin3-glycoside) in callus cultures of *Dimorphotheca sinuate* [73].

Maharik N. et al. used stems and leaf explants of *Crataegus sinaica* in-vitro regenerated shoots for initiating callus cultures. Stem and leaf were inoculated in vials containing 20mL MS media containing 2,4 dichloro-phenoxy acetic acid along with KIN. Carbon source was set to be 3% sucrose with pH 5.7 followed by autoclaving at 121°C (20 minutes) [74]. Cultured tissues were sub-cultured monthly in new fresh medium. Calluses of both stem and leaf were shifted to MS medium which contained varying sources of cytokinin for anthocyanin production. Vials containing culture were capped and incubated. Dry and fresh weight of anthocyanin in callus was measured at intervals of 30 days [74].

They observed that stem and leaf explants had produced max callus on basis of dry and fresh weight on Murashige and Skoog (MS) media containing 1mg/L kinetin and 1mg/L 2,4-D. High concentration benzyl adenine (BA) and low concentration 1-napthaleneacetic acid (NAA) contained media produces anthocyanin showing pale-medium pink colored pigmentation from fifth week of explant inoculation. The anthocyanin produced from *C. sinaica* stem and leaf callus cultures on MS media containing 0.5mg/L NAA and 1mg/L kinetin after 42 days was 13.39µg/g and 12.77µg/g, respectively. Increased NAA concentration only causes increase in weight of callus instead of increasing accumulation of anthocyanin. Capability of stem tissues for production of anthocyanin seems to be lower than that of leaf explants when placed in same media containing same components [74].

3.3. Colchicine

Colchicine is an example of tri-cyclic toxic alkaloid. It is Food and Drug Administration approved alkaloid used for treating Mediterranean fever, gout [75], Behçet's disease and pericarditis [76]. It prevents microtubule growth and interacts with dimers of tubulin. it is also responsible for polyploidy induction in plants [77]. It boosts mitotic karyokinesis in white blood cells. Chromosomes number doubles as a result of colchicine action that prevents formation of spindles dividing cell metaphase [78]. Colchicine is effective in degranulation of lysosome and increasing cAMP level [79].

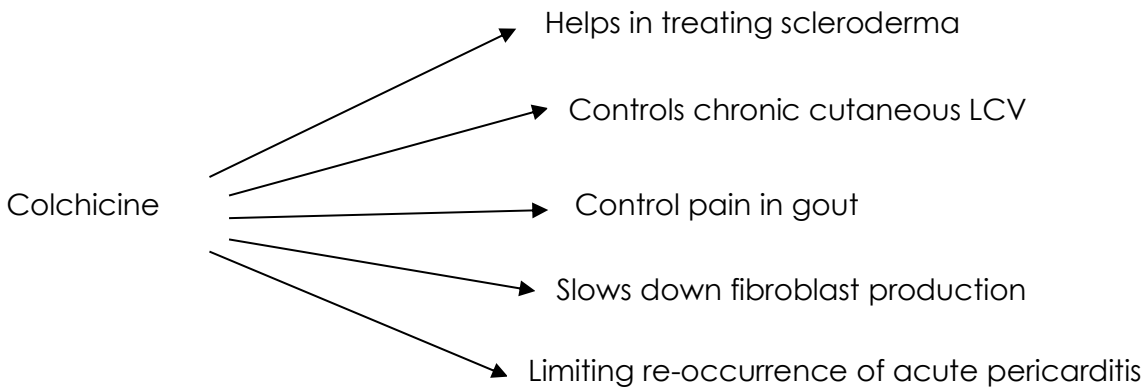


Figure 7. Medicinal importance of Colchicine

The main components of colchicine include three rings: A ring that is tri-methoxyphenyl, B ring which is 7 membered rings containing acetamide at 7th position and C ring that is tropolonic ring. Molecular formula of colchicines is $C_{22}H_{25}NO_6$ with mol. wt. equals to 399.43 Da [80]. Colchicine is water soluble and sparingly soluble in ethers [81]. It is stored in bottles with dark color. 0.8 mg/Kg colchicine is lethality dose but the concentration below this value have also proved lethal in some cases [82].

Colchicum species and *G. superba L* are the main sources of colchicine [81]. Seeds and rhizomes of *G. superba* contains higher concentration of colchicine [83] of approximately 0.7–0.9% [84]. Colchicine is also obtained from *G. superba* adventitious, hairy root, and callus cultures but in low amount [85]. Highest concentration of colchicine in *C. autumnale L.* is in its seeds -0.5%-1.2% and in corm it is 0.6% [86].

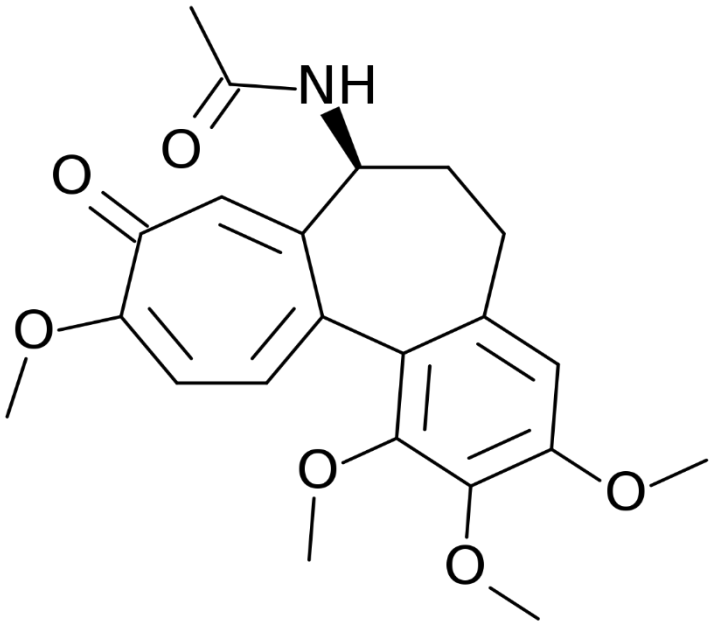


Figure 8. Structure of Colchicine [87]

Gloriosa superba L. rhizome were used as explant for tissue cultured produced of colchicine by Mahendran et al. [88]. They cultured the rhizome on media with 2,4-D and NAA and reported callus concentration to be 95.6% [88]. Mahendran et al. & Sen et al. and Shukor et al. showed in their results that NAA and 2,4-D supports the maximum callus production in cell cultures of *G. superba* [88], *Achyranthes aspera* [89], and *Polygonum minus* [90], respectively.

Mahendran et al. investigated different factors concentration on production of colchicine including: salicylic acid (SA), Yeast extract (YE), casein hydrolysate (CH) and silver nitrate ($AgNO_3$) [88]. All these factors increase the colchicine production. When cell suspensions are exposed to SA, both biomass and production of secondary metabolites increases. SA at 27.624mgL−1 concentration was found to be optimal dose for enhanced production of secondary metabolites in *G. superba* cell suspension culture gown for 30days period i-e: 8.149 mgg−1 dry weight (DW). YE at 300mgL−1 dose produced highest rate of colchicine content (7.813mgg−1 DW) for 15days of exposure in suspension culture [88].

Ghosh et al. [91] reported that the accumulation level of colchicine content was 5-fold higher in root cultures of *G. superba* grown at 2.5gL−1 CH concentration [91]. Flower and seed contain highest content of colchicine. It presents 50%-70% of alkaloid existing in plants [92]. Coumaric acid increases the colchicine content in *C. autumnale L.* callus tissues and that of *G. superba L.* root and callus tissues according to Aroud [92]. Tyrosine and phenylalanine were used for enhancing colchicine accumulation in callus tissues of *G. superba L.* by Sivakumar et al. [93]. Serine, mannitol, and phenylalanine increases the colchicine content in *G. superba* root cultures [93].

3.4. Ginkgolides

Ginkgolide is a sequestered and filtered product derived from the leaves of a medicinal plant named *Ginkgo biloba*. The leaf of *Ginkgo biloba* plant generally possesses flavonoids. For example, myricetin, terpene trilactone, and quercetin [94]. These metabolites are considered efficient antagonists of the (Platelet activating factor) PAF receptor [95]. Though, it is not present in large amounts in the roots and leaves of *Biloba.* Moreover, it has also been verified from different studies that climate, location, and periodic variations may distress its production [96, 97]. The potent compounds of this plant extract have remarkable advantages; such as it overcomes the formation of the blood clot, in an anaerobic condition, it shields the nerve cells from damage and strengthens the capillaries walls.

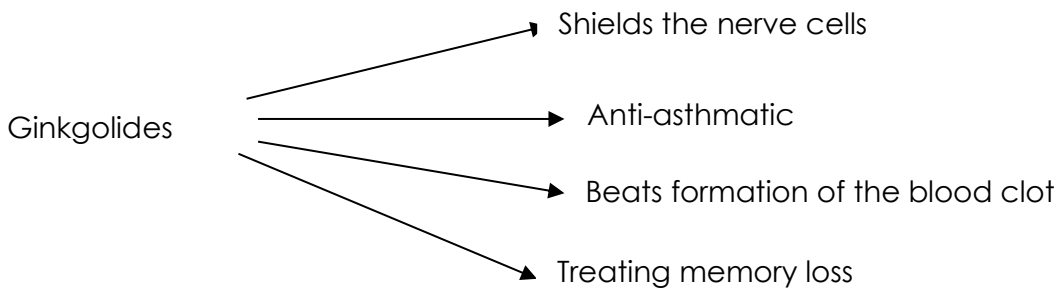


Figure 9. Medicinal importance of Ginkgolides

The extracts of its leaves have great use in treating memory loss disorders particularly complications regarding attention and dementia, moreover, having characteristics of neuroprotective by enhancing the mental capacity of Alzheimer's patients [98]. The

fragments of this useful explant are supplemented with curative agents for wounds, anti-oxidants [99], de-activation of free radicals [100], and antiasthmatic [101]. Ginkgolides belong to di-terpenoids class. They are di-terpene tri-lactones containing tertiary butyl group.

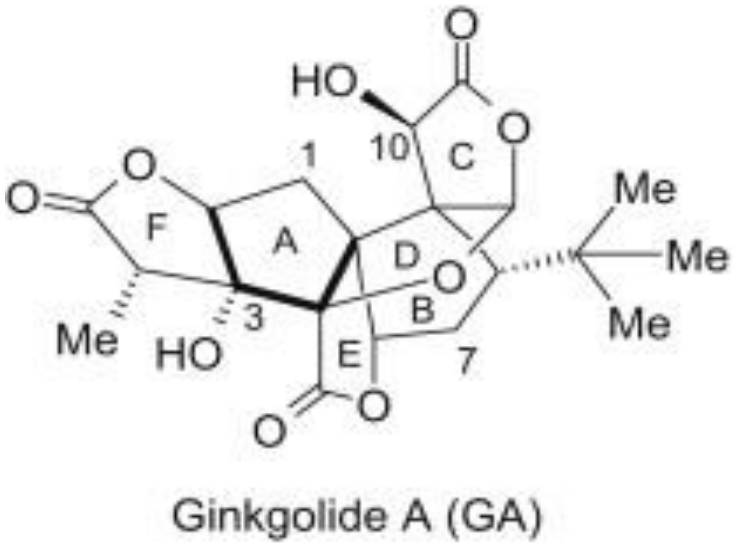


Figure 10. Structure of Ginkgolide A [102]

There are several types of research carried out to develop ginkgolides in the *in-vitro* condition by utilizing the cell cultures (undifferentiated) of *Ginkgo biloba*. It is ordinarily known as ginkgo. It has been listed in the category of "living fossil' and characterizes as a most valuable plant for manhood for more than two thousand years. One of the most vented plants from the category of medicinal plants in the world [103]. However, a very minute quantity of ginkgolide B was detected in plant tissue cultures. In comparison to it, the differentiated tissue cultures had shown amazing results. The studies have discussed that there are other great substitutes to develop the secondary metabolites which are primarily linked with cellular variations; specifically, the hairy root cultures [104].

A study carried out by Carrier and his co-workers exposed growth and nutrient uptake in the extracellular environment via cell suspension cultures of *G. biloba* in the shake flasks [105]. Another study had elucidated the formation of ginkgolides by culturing of

explant cells in the bioreactors. The yield of this secondary metabolite was observed from the *Biloba* at 19, 7, and 17 median concentrations [105]. Tissue culture method had been employed for leaves of ginkgo by many researchers in various studies to narrow down the metabolism in order to determine that how this particular metabolism synthesizes biochemically. Ahead of time, Koji Nakanishi et al. successfully inspected the biogenesis of ginkgolide B in the propagated sprouts [106].

Similarly, in around 1993 a remarkable study had occurred that revealed which culture support the formation of Ginkgolide greatly and which do not result in its great production; both callus and suspension culture resulted in its less production, though organ cultures significantly resulted in the development of secondary metabolite-ginkgolide [96]. To attain the highest yield of ginkgolide B, Jeon MH et al. have utilized both Murashige and Skoog (MS) and Schenk and Hildebrandt (SH) media to determine the role of both of these. The cell suspension culture showed a drastic reduction in production after attaining the supreme formation on the 13th day of subculturing by Jeon MH et al. The results of their study elucidated that synthesis of GKB was maximum in MS media whereas the SH media showed the endorsed cell growth [107]. The MS media got modified with the additional contents such as 30 g/L $C_{12}H_{22}O_{11}$ and K_2SO_4 (1.25 mM) along with NH^{+4} to NO^{-3} of 1:3 molar ratio, plus 1.0mg/L and 0.1mg/L of $C_{12}H_{10}O_2$ and kinetin, respectively [107].

Kang et al. conducted a study to determine elicitors effect on the ginkgolides production from *Ginkgo biloba* cell suspension culture. For this purpose, they selected salicylic acid (SA) and methyl jasmonate (MJ) as an elicitors. They observed an increase in ginkgolide B concentration up to 6.25 folds when cultures were treated with 1.0mM MJ while when treated with same concentration of SA an increase in ginkgolide B concentration was 6.1 folds. These results indicate that exposure time and concentration of both elicitors enhances the amount of ginkgolides produced by cell suspension cultures of *Ginkgo biloba* [108].

3.5. L-DOPA

It is one of the most distinguished secondary metabolites of intermediary pathways among the higher plants. Melanine, alkaloids as well as betalain are considered its

pioneers. It can be isolated from a medicinal plant known as *Mucuna*. The Mucuna plant is generally used in Ayurveda (1500-1000 BC), a former western medical science practiced in India from the Vedic times. A study carried out in 1996 revealed that this plant contains L-3,4-dihydroxyphenylalanine [109]. It has also been used in Ayurveda [110]. The bean, when added to scorpion stings as a paste, is believed to absorb the venom, moreover, it acts as a potent aphrodisiac and has been used to cure nervous disorders and arthritis [111]. One study has highlighted its role in controlling the blood glucose level in diabetic patients; having properties of acting as anti-cancer, it has great demand in the Indian Territory. Likewise, aloe vera, this plant has remarkable anti-microbial properties; most people use its powder to minimize the acne and scars on their skin. To control seizures and central nervous system disorders especially epilepsy this metabolite has great applications [112]. L-Dopa is a dominant neurotransmitter, which is thought to be partly responsible for the toxicity of its seeds [113]. Its seeds tend to kill the parasitic worms. In the Indian continent, it is also being used to cure male infertility. The intermediary metabolism of L-Dopa shows that whenever tyrosine gets restricted to convert into L-Dopa the amount of dopamine in the brain gets diminished which can be resulted from many factors including the absence of a rate-limiting enzyme. For normal biological processes, it must undergo a transition to dopamine for restoring normal brain functions [114].

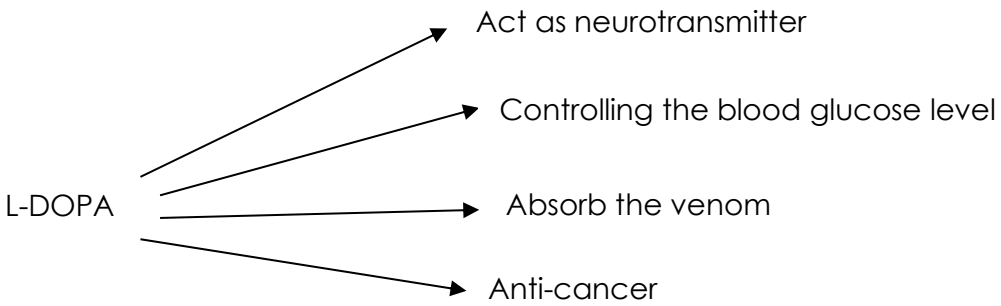


Figure 11. Medicinal importance of L-DOPA

Particularly in animals, this metabolite is an active substance of catecholamines, having the effective characteristic of the drug against Parkinson's disease (a chronic debilitating condition related to the insufficient amount of dopamine in the brain). Due

to the broad application of this therapeutic approach, a large amount of L-DOPA has been demanded at a cost-effective market, and cell cultures were introduced as a substitute method for its enhanced manufacturing [115].

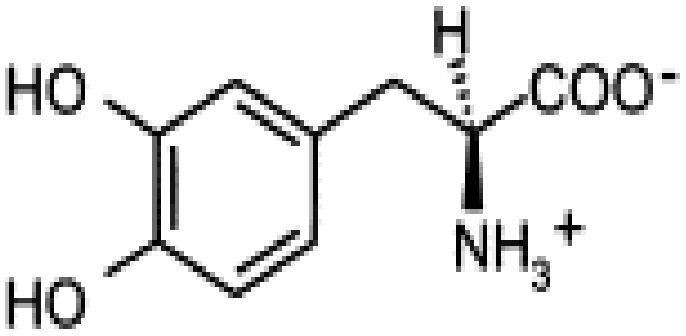


Figure 12. Structure of L-DOPA [116]

Mucuna is a standard plant been used in traditional Ayurvedic Indian medicine, moreover, the legume seeds of this plant result in L-Dopa from the non-protein amino acid [112]. The study that took place in 1976 described that medium with the considerably highest amount of 2,4-D mounts up L-Dopa (25mg/L) in callus culture of *Mucuna* [117]. In an experiment, L-Dopa tends to be developed from tyrosine when the cultured cells of explant halted in the Ca-alginate gels, and just after the arrest in it the cells of the *Mucuna* plant release L-Dopa in the medium [118]. Though, the accumulation of Ca into the medium ultimately results in suppression of metabolites conversion as well as its release in the media too. The cell suspension cultures normally result in the yield of L-Dopa (0.2 to 2%) [119].

The formation of secondary metabolites is contributed by various parameters that play a significant role in the efficient end-product development in culture. The major contributor in enhancing the production of L-Dopa in cell suspension culture of medicinal plant (*M. pruriens*) is the source of Nitrogen and illumination. The biogenesis of this particular metabolite gets affected by concentration as well as the nature of the N_2 source in the medium [120].

The inhibition of its production can be observed by the addition of an organic compound known as 2, 4-dichlorophenoxyacetic acid, moreover, phosphate and sucrose as a carbon source disturb its formation in cell suspension cultures. Defined nutrients with proper selection can operate the synthesis of plant products through cell suspension cultures. This metabolite tends to accumulate intra-cellularly by the cell suspension cultures [120].

3.6. Diosgenin

One of the most enormously significant metabolites for the pharmaceutical industry is diosgenin. Diosgenin is found to carry to be effective in prevention of tumors [121], osteoporosis [122], coronary conditions [123], atherosclerosis [124], diabetes mellitus [125], and skin disorders [126]. It is gradually being studied in the treatment of neurological disorders [127], such as, Parkinson's and Alzheimer's [128, 129].

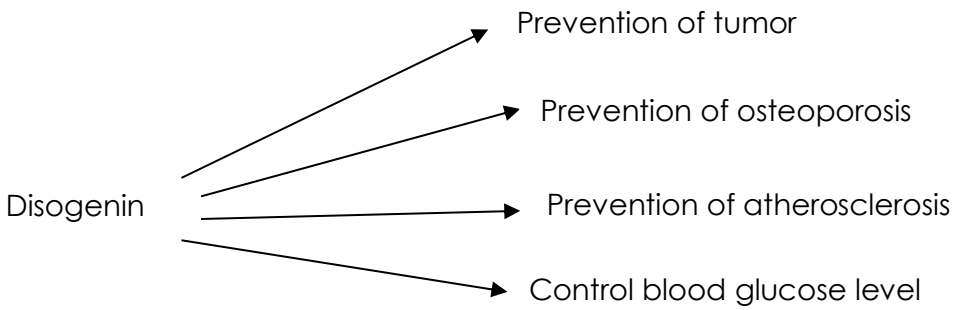


Figure 13. Medicinal importance of Diosgenin

It acts as a precursor in the synthesis pathway of steroidal drugs [130]. Diosgenin, steroid sapogenin, belong to class of steroids. $C_{27}H_{42}O_3$ is mol formula of diosgenin with mass 414.62 Da. It appears to be needle like crystals of white color. It is highly stable [131].

The *Dioscorea* genus has recently been a very common source of steroidal saponins such as diosgenin. The immobilized cell cultures of *Dioscorea* developed that particularly utilized the webbed organic polymer named polyurethan foam; it activated the yield of diosgenin (25%) Inclusive of 40% cellular concentration [132]. On the other hand, approximately 8% highest yield and regular output of 7.3mg/L of diosgenin were observed in batch culture of *D. deltoidea*. Along with the yield, the

levels of carbon and nitrogen were also investigated which can greatly enhance the development of diosgenin in culture [133].

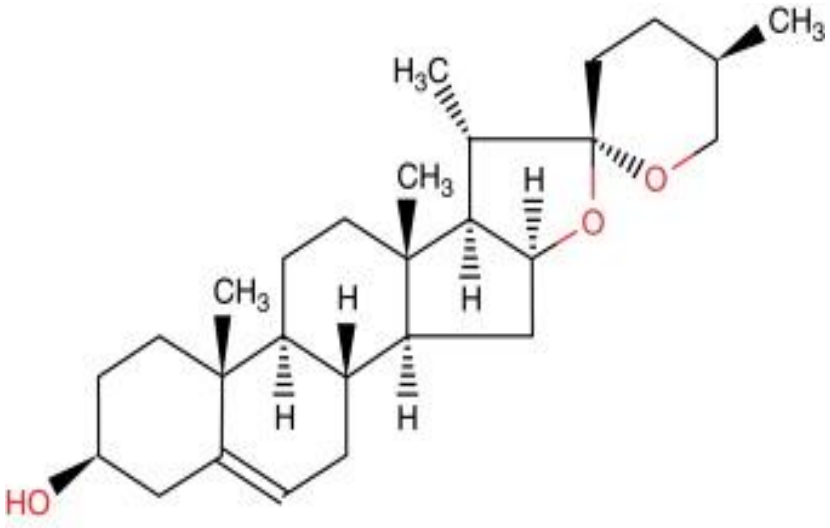


Figure 14. Structure of Diosgenin [134]

Researchers employed the *Dioscorea doryophora's* cell suspension culture to intensify the yield of disogenin. Moreover, the use of micro tuber and cell suspension derived from stem-node was analyzed to compare the amount of secondary metabolite outcome. As 6% sucrose is considered as an optimum quantity for growth; though diosgenin observed in quite a good amount in cultures containing 3% of sucrose only. The results of high-performance liquid chromatography shown the production of diosgenin by both of the suspension cultures (Microtuber and stem-node), but 0.3% outcome observed from stem-node whereas approximately 3% of diosgenin formation from micro-tuber, moreover, it is also investigated that cell suspension culture is also a useful technique to get diosgenin through the medicinal plant [135]. Ciura J et al. have conducted study to evaluate the importance of carbohydrates for production of disogenin. For this, they implanted sterilized Trigonella foenum-graecum seeds in MS media with varied concentration of sucrose (one with 3% and the other with 1% sucrose). After growth of plant, the disogenin concentration was observed by ultra

performance liquid chromatography. The media containing high concentration of carbohydrates resulted in high amount of disogenin production [136].

4. Conclusion

For the commercial development of medicinally important compounds, *in vitro* proliferation of medicinal plants with supplemented bioactive compounds as well as cell culture approaches for selective metabolite processing have proven extremely useful. Plant cell culture advances could pave the way for new ways to treat even sporadic or exotic plants. Complete insight understanding of the biosynthetic mechanisms of the target compounds in plants and cultures is also still very basic, and hence procedures are needed to establish knowledge-based on molecular as well as cellular levels to enable the sustainable use of medicinal plants for future generations.

Author Contributions

Conceptualization, G.Z.J., S.A.,: validation, G.Z.J.; formal analysis, S.A., A.S., F.N., A.Y., G.Z.J.; investigation, T.A., N.A.; writing—original draft preparation, S.A.; writing—review and editing, G.Z.J., A.S., F.N., A.Y.,; supervision, G.Z.J. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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