Plant Archives Vol. 20, Supplement 2, 2020 pp. 2683-2689 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ABIOTIC ELICITATION: A TOOL FOR PRODUCING BIOACTIVE COMPOUNDS

Biji A Varghese, Nisha Kumari, Sruchi Devi, Minhaj Ahmad Khan, Surender Jangra, Arvind Kumar*

Department of Biochemistry, School of Bioengineering and Biosciences,
Lovely Professional University, Phagwara, Punjab-144411, India.
* Author for correspondence: arvind_ldl@rediffmail.com

Abstract

Metabolism is the process by which plants produce various products and intermediates known as the ‘metabolites’ of plants. Primary metabolites such as carbohydrates, lipids and amino acids are produced. In addition to this, higher plants also synthesize various low molecular weight compounds called secondary metabolites that establish the defense mechanism in them. These secondary compounds are important sources for various industries like pharmaceuticals, food additives, flavor and fragrances, dye and pigments, pesticides and industrially important biochemicals. Most of the drugs that are sold now days are modified forms of the natural compounds. This utilization of natural substances has gained much attention over the past decades, particularly in the possibility of altering the production of bioactive plant metabolites. Since ancient time these metabolites have played a significant role in the field of medicine. In Ayurveda, Unani and other traditional medical systems, plants were directly used as food or herbs to treat various diseases for many years. However, these secondary constituents are produced often very low in the plant itself and their accumulation depends on some particular conditions, or growth stage, or may be on stress. *In vitro* culture of plant cells respond to microbial, physical, or chemical factors called “elicitors”. This process of induced production of secondary metabolites by plants for their defence is called elicitation. “Elicitors” are termed as the chemicals (biotic or abiotic) that gets triggered by a stress induced response or stimulate the defense mechanism in plants which therefore accumulates secondary metabolites, hence it can be a one of the best approaches for enhancement of production of secondary metabolites.

***Keywords*:** Metabolites, secondary metabolites, medicines, elicitation.

Introduction

A major portion of our everyday meal constitutes plants. Researchers are intensively studying the constituents of plants and their various nutritional importance from decades. Metabolism is the process by which plants produce various products and intermediates known as the ‘metabolites’ of plants. Primary metabolites such as carbohydrates, lipids and amino acids are produced. In addition to this, higher plants also synthesize various low molecular weight compounds called secondary metabolites that establish the defense mechanism in them (Amit *et al*., 2014; Gupta *et al*., 2014; Sirohi *et al*., 2014a; Saraswat *et al*., 2014; Wang and Wu, 2013).

There are three main groups of secondary metabolites on the basis of their biosynthetic origins. They are terpenoid, flavonoid and phenolic compounds, and nitrogen-containing alkaloid and sulphur-containing compounds (Bharadwaj *et al*., 2018; Kaur *et al*., 2019; Mahmoud and Croteau, 2002; Prasad *et al*., 2011; Sidana *et al*., 2012; Saxena *et al*., 2016). According to their specific structure, we have glycosides, tannins and saponins as well (Prasad, 2010; Sirohi *et al*., 2014b).

These secondary compounds are important sources for various industries like pharmaceuticals, food additives, flavor and fragrances, dye and pigments, pesticides and industrially important biochemicals (Murthy and Paek, 2014). Most of the drugs that are sold nowadays are modified forms of the natural compounds. This utilization of natural substances has gained much attention over the past decades, particularly in the possibility of altering the production of bioactive plant metabolites (Canter and Chhikara *et al*., 2018a; 2018b; 2019; Ernst, 2005; Kumar *et al*., 2019; Pandey *et al*., 2016; Sidana and Joshi, 2013; Thomas *et al*., 2011).

Since ancient times these metabolites have played a significant role in the field of medicine. In Ayurveda, Unani and other traditional medical systems, plants were directly used as food or herbs to treat various diseases for many years (Bhat *et al*., 2017; Dhawan and Gupta, 2017; Dohroo *et al*., 2016; Fabricant and Farnsworth, 2001; Mukherjee *et.al.,* 2007; Murali *et al*., 2017). The discovery of many bioactive compounds such as Taxol, Morphine, Metformin etc has witnessed plants to be an important source for drug discovery. These discovered molecules have been isolated to treat diseases like obesity, cancer and diabetes (Saklani and kutty, 2008).

The vast applications of these secondary compounds made them important for mass production. It was estimated that the international market value of naturally derived drugs was 18 billion dollars in 2005 and in 2011, it increased to more than 26 billion dollars (Saklani and Kutty, 2008). However, these secondary constituents are produced often very low in the plant itself and their accumulation depends on some particular conditions, or growth stage, or may be on stress. Also, some of these compounds are specific to a particular plant family or genus or even a species. Most of the important plants of medicinal value can only be cultivated in particular climates like tropic or subtropics regions. There are some plants that are slow in growing and some are even hard to cultivate, thus they are required to be harvested from the wild that can extinct those plant species (Alfermann and Petersen, 1995; Verpoorte *et al*., 2002). For such reasons, an approach that uses cell or tissue cultures of plants is being preffered as an alternative method for the production of these important bioactive compounds from plants.

This project is undertaken to study, the enhancement of secondary metabolites using abiotic elicitation in holy basil (*Ocimum tenuiflorum*) as, not much research has been carried out on the same. These plants are well known for their therapeutic potentials (Prakash *et al*., 2005). *O. tenuiflorum* is an herb with strong aroma cultivated throughout India. In Hindi it is called 'Tulsi' and in English 'Holy Basil'. In traditional medical systems its different parts (leaves, stem, flower, root, seeds and even whole plant) were recommended for the treatment of diseases such as bronchitis, malaria, diarrhea, skin diseases, arthritis, chronic fever and insect bite and many other. *O. tenuiflorum* has also been thought to possess properties such as anticancer, anti-diabetic, anti-microbial, cardioprotective, analgesic etc. Many researchers have reported that different chemical constituents are possessed in all members (even the close ones) of the same genus *Ocimum*. Razdan (2003) has reported that *O. tenuiflorum* can cross-pollinate with different plants of the same genus due to which some plants may not be of true-type. Hence, if some genetic variations are present, there would be different chemical constituents found in the plants. Here is the situation to apply plant *in vitro* culture that will help to solve this problem because the tissue culturing of plants will produce the identical offspring to that of the parent plant.

For multiplication and extraction of secondary metabolite we can estabalish cell and tissue culturing of plants under sterile conditions from seeds or explants (leaves, stems, roots, and meristems). Further, various strategies can be used to increase the production of bioactive compounds like precursor feeding, media optimization, in situ product removal, elicitation, immobilization etc (Verpoorte *et al*., 1999; Zhang and Furusaki, 1997). Among all these strategies, elicitation is proved to be one of the efficient way to enhance the production (Verpoorte *et al*., 1999). *In vitro* culture of plant cells respond to microbial, physical, or chemical factors called "elicitors". This process of induced production of secondary metabolites by plants for their defence is called elicitation. "Elicitors" are termed as the chemical (biotic or abiotic) that gets triggered by a stress induced response or stimulate the defense mechanism in plants which therefore accumulates secondary metabolites (Radman *et al*., 2003).

Plants are vulnerable to various environmental stresses that restrict their growth and yield causing a huge loss to agricultural production across worldwide (Kapoor *et al*., 2018; Madakemohekar *et al*., 2018; Pooja and Bhupendra, 2018; Shao and Chu, 2008 Siddique*et al*., 2018). One such important factors affecting growth of plants and their secondary plant products is salt stress (Nikolova and Ivancheva, 2005). Salinity has adverse effects on plant's physiological and metabolic activities. It results in late germination, stunted growth, poor quality and reduced productivity (Muhammad and Hussain, 2010). Also there is decrease in the rate of photosynthesis and respiration due to salt stress.

Through a process called 'metabolism', plants can produce different bio-active constituents. Thus they have been considered as the source for many important drugs. Plant cell carries out two major metabolisms namely, primary and secondary. The production of proteins, lipids, polysaccharides by utilizing sugars, amino acids, fatty acids and nucleotides is by primary metabolism. Plant secondary metabolism is related with the defence mechanism in plants which activates only during specific growth stages or during stress times, at the time of nutrient deficiency or microbial attacks (Yazaki *et al*., 2008).

Secondary metabolites of plants

Secondary compounds are the organic compounds that have no role in the life processes of plants that produce them but, they do provide them survival functions. These compounds are an important feature of all plants because these bioactive molecules produce defense mechanism in plants against different microbes and herbivores (Sharma *et al*., 2017; Stamp and Nancy, 2003). The various functions served by them are as follows:

(a) They can be used as transporting agents of metals;

(b) They act as protective agents against insects, microbes and higher animals;

(c) They can act as sexual hormones;

(d) They can also act as differentiation effectors.

Some compounds, including antibiotics helps in formation of spore and stop the germination process. Thus, the secondary compounds can perform functions like:

(a) Slowing down of spore germination till a favorable condition exists

(b) Protecting dormant spores from amoebae; and

(c) Cleansing of competitive microbe environment at the time of germination (Demain and Fang, 2000).

The extraction and purification of these molecules are difficult because they are often specific to species or genus and also produced in very low amounts. They are very high value products and have huge commercial applications in industries like food additives, biopesticides, colors, fragnances, medicines, agrochemicals, etc (Cusido *et al*., 2013).

Classes of secondary metabolites

The plant secondary metabolites can be grouped as terpenoid, alkaloid and phenolic (Verpoorte, 1998). According their specific structure, we have glycosides, tannins and saponins as part of them.

Terpenoid

Terpenoid is the largest group of plant secondary metabolites. They are volatile compounds. They are made of condensation of isoprene units (C5). They are classed by five carbon units found in the core structure (Mahmoud and Croteau, 2002). Isoprene is a gas released by the leaves that are produced in the chloroplasts. It protects the plants from heat.

The fragrances in the plants are due to the presence of essential oils. Some plants use the scent for the protection from herbivores and dangerous pathogens. Humans use essential oils for aromatherapy to enhance mood. It also helps in the functioning of mental and are thought to have various other benefits like oral bacteria potency, wide use in mouthwashes that are antiseptic, for skin problems and respiratory diseases.

Another type is taxol, which have great importance in the medical field to treat breast and ovarian cancers. The bark of Pacific yew tree produces taxol in very few amounts that its synthesis killed the tree. Therefore, other sources were found like a fungus on tree and needles in European yew.

Rubber is the last type which is also the largest of terpenoids because it contains 400 isoprene units. Rubbers have a large number of applications since decades. Today, rubbers are used in shoes, tires, erasers, gloves and spandex.

Phenol and Flavonoid

Phenolics have one aromatic ring with hydroxyl groups' attached with it. It present throughout the plant kingdom (Strack, 1997). Phenolics perform functions like they prevent block activity of enzyme and division of cells (Shapiro *et al.*, 1994).

Flavonoids are the first group of phenols. They are found in vacuoles and are water-soluble. In plants, they perform functions like UV protection, pigmentation, disease resistance (Koes *et al.*, 1994). Flavonoids are further grouped into three classes that are anthocyanin, flavone and flavnol. Anthocyanin ranges from red color to blue and purple color. The pH of environment decides its color. They can be found in grapes and berries. It can be used to treat heart diseases, diabetes and cancer. They are found in skincare products also to minimize process of aging. The other groups are flavones and flavnols that have white or yellow pigments. As a group, the phenols help in pollination of plants.

Salicylic acid is another important compound, which is obtained from bark of willow tree. It is an active ingredient in aspirin. It has been used to effectively in the treatment of aches and fevers and has cosmetic applications.

Lignin is the last compound that is present in plant structure. It provides stiffness or strength to plant cell walls. It does not allow water to enter cell wall and therefore prevent attacks by fungus.

Nitrogen containing compounds: Alkaloids

Plant secondary metabolites have large amounts of nitrogen in their structure. Alkaloids form part of this category which is known for its anti-herbivore property. They are of great interest because of their medicinal properties. They can also be highly toxic to the humans.

Morphine was the first alkaloid. Morphine can be found in plant *Papaver sonniferum*, or opium poppy. It is used to treat pain and suppress cough.

Cocaine is another compound. It may be dangerous if taken in large amounts. It can be used as an anesthetic. Its derivatives are dangerous when consumed on regular basis and prove to be deadly.

Caffeine is a known compound. It protect its source plant that is tea,coffee and cocoa. Humans use it to stay alert but their high concentration is very toxic It protect the plant by inhibiting germination of other plants in that area and in humans, caffeine is thought to minimize diabetes and heart problems.

***In vitro* production of secondary compounds**

The production is often low (> 1 percent dry weight) which is dependent on geo-climatic and plant growth stage (Rao and Ravishankar, 2002). Accumulation of such metabolites is directly dependent on external and internal stresses. As a response to stress factors viz. mechanical injury, minerals in soils, microbial infections, UV radiation, and accumulation of secondary metabolites largely varies in terrestrial plants.

Most of the plants are difficulte to cultivate and hence are over-harvested (Rates, 2001). Secondly, the chemical production of these active compounds is not feasible for commercial production. In this situation, *in vitro* culturing of plant cell offers a best option for secondary compound production (Jian *et al.*, 2005).

Defence response produced by secondary substances: Elicitation concept

The induced production of metabolites by adding trace amounts of elicitors is called Elicitation. The substances that are introduced in small amounts to generate plant defense mechanism are called elicitors (Klarzynski and Friting, 2001). Elicitation is stated as the most preffered technique for increasing the productivity of desirable secondary substances *in vitro* (Namdeo, 2007).

Classification of Elicitors

Elicitors are classified as abiotic and biotic depending on their origin (Radman *et al.*, 2003).

Abiotic Elicitors

Abiotic elicitors are from non-biological origin (Gorelick and Bernstein, 2014). Salts such as aluminium chloride, calcium chloride, copper chloride, potassium chloride etc. can be used to elicit production in different plant species in culture systems (Verpoorte *et al.*, 2002).

Biotic Elicitors

These are substances of biological origin. They include polysaccharide originated from cell wall of the plant for example pectin and cellulose and microorganisms for example yeast, fungal extracts and bacterial extracts.

These are molecule of either pathogen or host origin which can generate defense responses in plant tissue. Biotic elicitors are recognized by unique receptors bound to the membrane of the cell. A signal transduction system transfers this stimulus, that produces changes and lead phytoalexins formation (Baenas *et al.*, 2014).

Effect of Abiotic Elicitors on the production of secondary compounds

The effects of abiotic elicitors are studied in the following section. Abiotic elicitors were not much used in past years as compared to biotic elicitors (Radman *et al.*, 2003). Studies have been done to observe the plant response towards different abiotic elicitors such as heavy metals, light, drought and many others (Rodziewicz *et al.*, 2014).

Physical factors

Physical factors such as ultrasound, light, osmotic stress, salinity, drought and thermal stress can be used.

Ultrasound

US act as an abiotic elicitor (Yu *et al.*, 2015). It was studied that US treatment increased the production of shikonin 2-3 times in *Lithospermum erythrorhizon* cultures (Lin and Wu, 2002). In *Panax ginseng* culture, US increased production of saponins (Lin *et al.*, 2001).

Light

It was observed that light increased the production of gingerol and zingiberene in culture of *Zingiber officinale* (Anasori and Asghari, 2008). Exposure light to light increased production of artemisinin in *Artemisia annua* (Liu *et al.*, 2002).

Osmotic stress

It is reported that osmotic stress influence growth of plants and secondary compound formation (Liu and Cheng, 2008). A study showed that in *Capsicum chinensis* cultures, the osmotic stress increased the productivity of capsaicin (kehie *et al*., 2014).

Salt stress

When plants are given salinity conditions they accumulate secondary compounds like terpene, alkaloid and phenol (Selmar, 2008). It was reported that *Bacopa monnieri* cultures produced bacoside A content in large amounts when given potassium chloride and calcium chloride treatments (Ahire *et al*., 2014). Similarly, when sodium chloride treatment was provided to *Nitraria tangutorum* culture, sitosterol content was increased (Ni *et al*., 2015).

Drought stress

Drought can limit the growth in plants as well as their reproductive development. All plant species posses different tolerance from drought stress. A study showed that when plants were provided drought conditions, it lead to accumulation of secondary substances (Al-Gabbiesh *et al*., 2015). Polyethylene glycol was treated with date palm callus to induce drought stress. With increase in PEG concentration there was an increase in the content of proline also (Al and Ibraheem, 2014).

Thermal stress

Extreme temperature conditions can restrict the growth in plants by affecting their metabolic processes (Xu *et al*., 2013). To induce callus tissues a temperature range of 17-25 degree Celsius is used (Rao and Ravishankar, 2002). A study reported that the anthocyanin yield in the culture of *Perilla frutescens* was maximum at temperature 25 degree Celsius (Zhong and Yoshida, 1993). Similarly, ginsenoside content was increased in *Panax quinquefolius* by 5 degree increase in temperature (Jochum *et al*., 2007).

Chemical Elicitors

Metals can be used to produce secondary substances in plants (Nasim and Dhir, 2010). It is thought to be an abiotic stress for plants due to their wide use in industries, agro-technology (Cai *et al*., 2013). A study reported that metals such as nickel, silver, iron and cobalt can elicit the secondary compound production in many plants (Wang and Wu, 2013). Cobalt was used in the culture of *Vitis vinifera* to increase production of phenolic acid (Cai *et al*., 2013).

Factors which influence elicitation

Some of the important factors that can affect the elicitor-plant cell interaction and hence the elicitation responses are as follows:

Elicitor specificity

Elicitors are specific to each plant culture. If we treat a particular culture with different elicitors also we will observe the accumulation of same compounds only. The induction kinetics or level of accumulation may vary with different elicitors but, the metabolite class depends on the plant species. A study reported that elicitation by biotic or abiotic compounds in the hairy root cultures of *Brugmansia candida* showed differences in induction kinetics and release of hyoscyamine and scopolamine levels (Pitta-Alvarez *et al.,* 2000).

Concentration of elicitor

The concentration of elicitor strongly affects the response intensity and dose effectiveness. The elicitor concentration may vary according to the plant species. A study reported that at a concentration of 0.5 percent sodium chloride productivity of ginseng saponin content increased 1.13 times the control value (Jeong and Park, 2007). Another report showed that yield of glycyrrhizic acid in *Taverniera cuneifolia* root cultures increased to 2.5 times when treated with MeJA of 100 microM concentration (Awad *et al* ., 2014).

Treatment interval

Each plant species require different time interval for maximum metabolite accumulation. It is generally preceded by an increased metabolic enzymatic activity. A study described that the anthraquinone content was doubled when *Pythium aphanidermatum* was used to treat *Rubia tinctorum* cell cultures which was preceded by an increase in the activity of isochorismate synthase (Van Tegelen, 1999).

Growth stage

The elicitor should be added during the log phase of growth when the activity of enzyme is maximum (Vasconsuelo *et al*.; 2003). Hence, an efficient response can be achieved.

Composition of medium

The growth factors in the medium also affect the elicitation. A study showed that if auxin is not provided in the medium, the carrot cells in the culture do not respond to elicitation (Kurosaki *et al*., 1985).

Light conditions

The light condition of culture also plays a major role. A study reported the production of hypericin in *Hypericum perforatum* L. cells showed higher cell growth under light than when incubated in the dark (Walker *et al*., 2002)

References

Ahire, M.L.; Laxmi, S.; Walunj, P.R.; Kavi Kishor, P.B. and Nikam, T.D. (2014). Effect of potassium chloride and calcium chloride induced stress on in vitro cultures of *Bacopa monnieri* (L.) Pennell and accumulation of medicinally important bacoside A. J Plant Biochem Biotechnol.; 23(4): 366–378.

Al-Gabbiesh, A.; Kleinwächter, M. and Selmar, D. (2015). Influencing the contents of secondary metabolites in spice and medicinal plants by deliberately applying drought stress during their cultivation. Jordan J Biol Sci.; 8(1): 1-10.

Alfermann, A.W. and Peterson, M. (1995) Natural product formation by plant cell biotechnology – Results and perspectives. Plant Cell Tiss Organ Cult 43: 199–205.

Al-Khayri, J.M. and Ibraheem, Y. (2014) In vitro selection of abiotic stress tolerant date palm (*Phoenix dactylifera* L.): A review. Emir J Food Agric; 26(11): 921-933.

Amit, K.; Chiang, P. and Chen, S. (2014). Interrelationship between secondary metabolites of cereals and agronomic practice. International Proceedings of Chemical, Biological and Environmental Engineering (IPCBEE), 79: 11-14.

Anasori, P. and Asghari, G. (2008). Effects of light and differentiation on gingerol and zingiberene production in callus culture of *Zingiber officinale* Rosc. Res Pharm Sci.; 3(1): 59-63.

Awad, V.; Kuvalekar, A. and Harsulkar, A. (2014). Microbial elicitation in root cultures of *Taverniera cuneifolia* (Roth)Arn. for elevated glycyrrhizic acid production. Industrial Crops and Products. 54: 13–16.

Baenas, N.; Garcia-Viguera, C.; Moreno, D.A. (2014). Elicitation: A tool for enriching the bioactive composition of foods. Molecules, 19: 13541–13563.

Bhardwaj, S.V. (2018). Antimicrobial Activity and Molecular Docking Studies of a Sesquiterpenoid Alcohol from Leaf Solvent Extracts of Juniperus communis L. International Journal of Green Pharmacy (IJGP), 12(02): 22-28.

Bhat, A.A.; Mayirnao, H.S. and Fayaz, M. (2017). Paris polyphylla Smith–A critically endangered, highly exploited medicinal plant in the Indian Himalayan region. Journal of Chemical and Pharmaceutical Sciences July-September, 10(3): 1202-1210.

Cai, Z.; Kastell, A.; Speiser, C.; Smetanska, I. (2013). Enhanced resveratrol production in *Vitis vinifera* cell suspension cultures by heavy metals without loss of cell viability. Appl Biochem Biotechnol. Sep; 171(2): 330-40.

Canter, P.H.; Thomas, H. and Ernst, E. (2005) Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. Trends Biotechnol.; 23: 180–185.

Chhikara, N.; Devi, H.R.; Jaglan, S.; Sharma, P.; Gupta, P. and Panghal, A. (2018a). Bioactive compounds, food applications and health benefits of Parkia speciosa (stinky beans): a review. Agriculture & food security, 7(1): 46.

Chhikara, N.; Kaur, R.; Jaglan, S.; Sharma, P.; Gat, Y. and Panghal, A. (2018b). Bioactive compounds and pharmacological and food applications of Syzygium cumini–a review. Food & function, 9(12): 6096-6115.

Chhikara, N.; Kushwaha, K.; Sharma, P.; Gat, Y.; & Panghal, A. (2019). Bioactive compounds of beetroot and utilization in food processing industry: A critical review. Food Chemistry, 272: 192-200.

Cusidó, R.M.; Vidal, H.; Gallego, A.; Abdoli, M.; Palazón, J. (2013) Biotechnological production of taxanes:A molecular approach. In Recent Advances in Pharmaceutical Sciences III; Muñoz Torrero, D.; Cortés, A.;Mariño, E.L.; Eds.; Transworld Research Network: Trivandrum, India, 91–107.

Demain, A.L. and Fang, A. (2000). The natural functions of secondary metabolites. Adv Biochem Eng Biotechnol. 69: 1-39.

Dhawan, D. and Gupta, J. (2017). Research article comparison of different solvents for phytochemical extraction potential from datura metel plant leaves. Int. J. Biol. Chem, 11: 17-22.

Dohroo, A.; Karnwal, A. and Ghai, M. (2016). Recent developments in Neem (*Azadirachta indica*–A. Juss) derived antimicrobial constituents for control of human and plant diseases–a review. In Annales Academiae Medicae Silesiensis, 70: 220-223.

Fabricant, D.S. and Farnsworth, N.R. (2001). The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect, 109: 69-75.

Gorelick, J. and Bernstein, N. (2014) Chapter five-Elicitation: An underutilized tool in the development of medicinal plants as a source of therapeutic secondary metabolites. In Advances in Agronomy; Sparks, D.L.; Ed.; Elsevier: Amsterdam, The Netherlands, 124: 201–230.

Gupta, M.; Gupta, A. and Gupta, S. (2014) Characterization of Secondary Metabolites via LC-MS Analysis of DCM Extracts of Solanum nigrum. Biosci. Biotechnol. Res. Asia, 11(2): 531-535.

Jeong, G.A. and Park, D.H. (2007). Enhanced secondary metabolite biosynthesis by elicitation in transformed plant root system. Applied Biochemistry and Biotechnology.;130: 436–446.

Jian Zhaoa, T.; Lawrence, C.D. and Robert, V. (2005). Elicitor signal transduction leading to production of plant secondary metabolites, Biotechnology Advances, 23: 283–333.

Jochum, G.M.; Mudge, K.W. and Thomas, R.B. (2007). Elevated temperatures increase leaf senescence and root secondary metabolite concentration in the understory herb Panax quinquefolius (Araliaceae). Am J Bot.; 94(5): 819-26.

Kapoor, D.; Bhat, A.A.; Bhardwaj, R.; Kaur, S. and Anand, R. (2018) Differential biochemical responses of Brassica juncea to cadmium stress: antioxidative defence system, photosynthesis and polyphenol synthesis. Poll Res.; 37: S135-S144.

Kaur, S.; Panesar, P. S.; Gurumayum, S.; Rasane, P.; & Kumar, V. (2019). Optimization of aqueous extraction of orevactaene and flavanoid pigments produced by *Epicoccum nigrum.* Pigment & Resin Technology. 48 (4): 301-308.

Kehie, M.; Kumaria, S. and Tandon, P. (2014). Osmotic stress induced—capsaicin production in suspension cultures of Capsicum chinense Jacq.cv. Naga King Chili. Bioprocess Biosyst Eng.; 37(6): 1055-63.

Klarzynski, O. and Friting, B. (2001). Stimulation of plant natural defenses. C. R. Acad. Sci. III, 324: 953–963.

Koes, R.E.; Quattrocchio, F. and Mol, J.N.M. (1994) The flavonoid biosynthetic pathway in plants: function and evolution. BioEssays, 16: 123–132.

Kumar, V.; Suri, S.; Prasad, R.; Gat, Y.; Sangma, C.; Jakhu, H.; & Sharma, M. (2019). Bioactive compounds, health benefits and utilization of Rhododendron: a comprehensive review. Agriculture & Food Security, 8(1): 6.

Kurosaki L.; Yeoman, M.M. (1985). Immobilized plant cells, in: M.M. Yeoman (Ed.) (1985) Plant cell culture technology, Berlin: Springer-Verlag, pp. 229-67.

Lin, L.; Wu, J.; Ho, K.P. and Qi, S. (2001). Ultrasound-induced physiological effects and secondary metabolite (saponin) production in Panax ginseng cell cultures. Ultrasound Med Biol. 27(8): 1147–52.

Lin, L. and Wu, J. (2002) Enhancement of shikonin production in single and two-phase suspension cultures of *Lithospermum erythrorhizon* cells using low-energy ultrasound. Biotechnol Bioeng.; 78(1): 81–88.

Liu, C.Z.; Guo, C.; Wang, Y. and Ouyang, F. (2002). Effect of light irradiation on hairy root growth and artemisinin biosynthesis of *Artemisia annua* L. Process Biochem. 38(4): 581-5.

Liu, C.Z. and Cheng, X.Y. (2008) Enhancement of phenylethanoid glycosides biosynthesis in cell cultures of *Cistanche deserticola* by osmotic stress. Plant Cell Rep. 27(2): 357-62.

Madakemohekar, A.H.; Prasad, L.C. and Prasad, R. (2018). Generation mean analysis in barley (Hordeum vulgare l.) Under drought stress condition. Plant Archives, 18(1): 917-922.

Mahmoud, S.S. and Croteau, R.B. (2002). Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends in Plant Science, 7: 366-373.

Muhammad, Z. and Hussain, F. (2010b). Effect of NaCl salinity on the germination and seedling growth of some medicinal plants. Pakistan Journal of Botany, 42(2): 889-897.

Mukherjee, P.K.; Rai, S.; Kumar, V.; Mukherjee, K.; Hylands,m P.J. and Hider, R.C. (2007). Plants of Indian origin in drug discovery. Expert Opin. Drug Discovery, 2: 633-657.

Murali, S.; Sriramachandran, R.; Nageswara Rao, G. and Basavaraju, R. (2017). Looking through the lens of a conservation biologist: life of medicinal plants in the eastern ghats of Andhra Pradesh, India. International Journal of Conservation Science, 8(2): 333-347.

Murthy, H.N.; Lee, E.J.; Paek, K.Y. (2014) Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. Plant Cell Tiss Org Cult. 118: 1-16

Namdeo, A.G. (2007). Plant cell elicitation for production of secondary metabolites: A review. Pharmacogn. Rev.1: 69–79.

Nasim, S.A. and Dhir, B. (2010). Heavy metals alter the potency of medicinal plants. Rev Environ Contam Toxicol.; 203: 139-49.

Ni, J.; Yang, X.; Zhu, J.; Liu, Z.; Ni, Y. and Wu, H. (2015). Salinity-induced metabolic profile changes in Nitraria tangutorum Bobr. suspension cells. Plant Cell Tiss Org Cult.; 122(1): 239-48.

Nikolova MT, Ivancheva SV. (2005) Quantitative flavonoid variations of *Artemisia vulgaris* L. and *Veronica chamaedrys* L. in relation to altitude and polluted environment. Acta Biol Szegediensis; 49:29-32.

Pandey, D.K.; Parida, S. and Dey, A. (2016). Comparative HPTLC analysis of bioactive marker barbaloin from in vitro and naturally grown Aloe vera. Revista Brasileira de Farmacognosia, 26(2): 161-167.

Pitta-Alvarez S.I, T.C. Spollansky, A.M. Giulietti, (2000) The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of Brugmansia candida, Enzyme and Microbial Technology 26 252-258.

Pooja, T.; & Bhupendra, K. (2018). Comparative assessment of drought and salt stress tolerance in three varieties of pearl millet (*Pennisetum glaucum* L.). Annals of Biology, 34(2): 119-125.

Prakash P, Neelu G. (2005) Therapeutic uses of Ocimum sanctum linn (tulsi) with a note on eugenol and its pharmacological Actions: a short review. Indian J. Physiol. Pharmacol. 49(2): 125-131

Prasad, D. (2010) Phenolic Glycoside from root of *Viburnum cylindricum*. Biosciences, Biotechnology Research Asia, 7(1), p. 501-502.

Prasad, D.; & Sati, S. P. (2011). A New Terpenoid 3R, 4R, 4aR, 5R, 6aS, 6bR, 12aR)-4a (Hydroxyl methyl)—2, 2, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 12b, 13, 14b-lcosahydropicene, 3, 4, 5, 10-tetraol from Scutellaria scandance. Oriental Journal of Chemistry, 27(4): 1767.

Radman R, Saez T, Bucke C, Keshavarz T. (2003). Elicitation of plants and microbial cell systems. Biotechnol Appl Biochem. 37(Pt 1): 91- 102.

Rao SR, Ravishankar GA. (2002) Plant cell cultures: Chemical factories of secondary metabolites. Biotechnol Adv.; 20(2): 101-53.

Rates S.M.K. (2001). Plants as sources of drugs. Toxicon (39): 603–613.

Ravishankar, G.A. and S.R. Rao,. (2000). Biotechnological production of phyto-pharmaceuticals. J. Biochem. Mol. Biol. Biophys.; 4: 73-102.

Razdan H. (2003) Colorimetrische Bestimmung des Rutingehalts inpflanzenextrakten und praparten mit Aluminiumchlorid inmit Eisessig - Pyridin gepufferter losung. Pharmazie 15: 33–38.

Rodziewicz P, Swarcewicz B, Chmielewska K, Wojakowska A, Stobiecki M. (2014) Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol Plant. 36: 1–19.

Saklani A, Kutty SK (2008) Plant-derived compounds in clinical trials. Drug Discov Today 13:, 161–171.

Saraswat, D.; Nehra, S.; Saxena, S.; & Singh, S. B. (2014). Ameliorative effect of Vitex peduncularis in neuroblastoma cells against oxidative stress under hypoxic condition. Journal of Herbal Medicine, 4(3), 115-126.

Saxena, S.; Shukla, D.; & Bansal, A. (2016). Expression of monocarboxylate transporter isoforms in rat skeletal muscle under hypoxic preconditioning and endurance training. High altitude medicine & biology, 17(1), p. 32-42.

Selmar D. (2008) Potential of salt and drought stress to increase pharmaceutical significant secondary compounds in plants. Agr Forest Res.; 58: 139–144.

Shao HB, Chu LY Jaleel CA. (2008) Water-deficit stress-induced anatomical changes in higher plants. C R Biologies; 331(3):215-25.

Shapiro S, Meier A & Guggenheim (1994) The antimicrobial activity of essential oils and essential oil components towards oral bacteria Oral Microbiology and Immunology 9, 202–208.

Sharma, N.R.; Meshram, V. and Gupta, M. (2017). Biological activities of novel in vitro raised Stevia plant. Asian J Pharm Clin Res, 10(8), p. 240-243.

Sidana, J.; Singh, S.; Arora, S. K.; Foley, W. J.; & Singh, I. P. (2012). Terpenoidal constituents of Eucalyptus loxophleba ssp. lissophloia. Pharmaceutical biology, 50(7), p. 823-827.

Sidana, J.; & Joshi, L. K. (2013). Recycle HPLC: A powerful tool for the purification of natural products. Chromatography Research International, Article ID 509812.

Siddique, A.; Kandpal, G.; & Kumar, P. (2018). Proline Accumulation and its Defensive Role Under Diverse Stress Condition in Plants: An Overview. Journal of Pure and Applied Microbiology, 12(3), p. 1655-1659.

Sirohi, S. K.; Goel, N.; & Singh, N. (2014a). Utilization of saponins, a plant secondary metabolite in enteric methane mitigation and rumen modulation. Annual Research & Review in Biology, 4(1), p. 1-19.

Sirohi, S. K.; Goel, N.; & Singh, N. (2014b). Influence of Albizia lebbeck saponin and its fractions on in vitro gas production kinetics, rumen methanogenesis, and rumen fermentation characteristics. ISRN veterinary science, Article ID 498218.

Stamp, and Nancy (2003) "Out of the quagmire of plant defense hypotheses".The Quarterly Review of Biology 78 (1):, 23 55.

Strack, D. Phenolic metabolism. (1997). In P.M. Dey and J.B. Harborne (eds), Plant Biochemistry.Academic Press, London, ,387–416.

Thomas, P.; Bansal, A.; Singh, M.; Shukla, D.; & Saxena, S. (2011). Preconditioning effect of cobalt chloride supplementation on hypoxia induced oxidative stress in male albino rats. Biomedicine & Preventive Nutrition, 1(2), p. 84-90.

Van Tegelen L. J. P, R.M. Bongaerts, A.F. Croes, R. Verpoorte, J. Wullems, (1999) Isochorismate synthase isoforms from elicited cell cultures of Rubia tinctorum, Phytochemistry 51, 263-269.

Vasconsuelo A, A.M. Giuletti, G. Picotto, J. Rodrıguez-Talou, R. Boland, (2003) Involvement of the PLC/PKC pathway in chitosan-induced anthraquinone production by Rubia tinctorum L. cell cultures, Plant Science 165 429-436.

Verpoorte, R. 1998 "Exploration of Nature's Chemodiversity: The Role of Secondary Metabolites as Leads in Drug Development." Drug Discovery Today 3 (5): 232-8.

Verpoorte, R.; Contin, A.; Memelink, J. 1999 Biotechnology for the production of plant secondary metabolites. Phytochem. Rev. 1, 13–25.

Verpoorte R, Memelink J (2002) Engineering secondary metabolite production in plants. Curr Opin Biotechnol 13:, 181–187.

Walker T.S, H.P. Bais, J.M. Vivanco, (2002) Jasmonic acid-induced hypericin production in cell suspension cultures of Hypericum perforatum L. (St. John's wort), Phytochemistry 60 289-293.

Wang JW, Wu JY. (2013) Effective elicitors and process strategies for enhancement of secondary metabolite production in hairy root cultures. Adv Biochem Eng Biotechnol.; 134: 55-89.

Xu Y, Du H, Huang B. (2013) Identification of metabolites associated with superior heat tolerance in thermal bentgrass through metabolic profiling. Crop Sci. 53: 1626–1635.

Yazaki, K.; A. Sugiyama, M. Morita and N. Shitan,. (2008) Secondary transport as an efficient membrane transport mechanism for plant secondary metabolites. Phytochem. Rev.;7:, 513-524.

Yu M, Liu H, Shi A, Liu L, Wang Q. (2016) Preparation of resveratrol-enriched and poor allergic protein peanut sprout from ultrasound treated peanut seeds. Ultrason Sonochem.; 28: 334-40.

Zhang W, Seki M, Furusaki S. (1997) Effect of temperature and its shift on growth and anthocyanin production in suspension cultures of strawberry cells. Plant Sci. 127(2): 207-214.

Zhong JJ, Yoshida T. (1993) Effects of temperature on cell growth and anthocyanin production in suspension cultures of Perilla frutescen. J Ferment Bioeng. 76(6): 530-1