PHYTOCHEMICAL ANALYSIS AND ELICITATION STUDIES IN *Plumbago zeylanica*, AN IMPORTANT MEDICINAL PLANT

To be submitted as Major report in partial fulfillment of the requirement for the degree of

Masters in Technology

In

Industrial Biotechnology

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled **"Phytochemical analysis and elicitation studies in *Plumbago zeylanica*, an important medicinal plant"**, which is submitted by Nazia Chaudhary (2K18/IBT/06) in the partial fulfillment of the requirements for the reward of the degree of Master of Technology, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by her under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honoring of any other degree.

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This is to certify that the thesis of Major Project II entitled "**Phytochemical analysis and elicitation studies in *Plumbago zeylanica*, an important medicinal plant**" in the partial fulfilment of the requirements for the reward of the degree of Mater of Technology, Delhi Technological University (Formerly Delhi college of Engineering, University of Delhi), is an authentic record of the my own work carried out under the guidance of my project supervisor **Dr. Navneeta Bharadvaja**, Asssistant Professor, Plant Biotechnology, Department of Biotechnology, DTU. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honouring of any other degree.

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ABSTRACT

Plumbagin a bioactive compound present in *Plumbago zeylanica* have several pharmacological properties like antibacterial, anticancer, anti-inflammatory etc. Due to its several pharmacological properties, this plant is being researched for high biomass production as well as achievement of maximum yield of bioactive compounds. This study examined the effect of abiotic elicitor on the production of plumbagin in *Plumbago zeylanica*. Elicitation is one of the approaches used for enhanced commercial production of secondary metabolite from plant cell culture system. In-vitro shoot of *plumbago zeylanica* cultured on MS medium supplemented with various concentration and types of abiotic elicitor and the elicitor which are used are salicylic acid, sodium acetate and silver nitrate (50 µM). Shoot were investigated for plumbagin accumulation using HPLC and it was found that elicitor significantly stimulated higher plumbagin content than the control treatment. Phytochemical analysis was also done for elicited and non- elicited shoot culture. Result indicate that by using elicitor increase in plumbagin production as well as phytochemical occur in shoot culture of *Plumbago zeylanica*.

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LIST OF ABBREVIATIONS

NBPGR- National Bureau of Plant Genetic Resources

HPLC- High performance liquid chromatography

BAP- 6-Benzylaminopurine

ºC- Degree Celsius

µg- microgram

µL- microliter

mL - milliliter

µM-micromolar

INTRODUCTION

India's civilization is immemorial and the country is known for rich resources of medicinal plants. Ayurveda, Homeo and Unani physicians utilize a number of plants and their components that have therapeutic effects. Among the various available antioxidants, the antioxidants derived from these medicinal plants have proven them as a better cure/ prevention for some of the human diseases.

Plumbago zeylanica L. Commonly known as Chitrak is widely used in India and around the globe. It is known by different name in different parts of the world viz. white leadwort or Ceylon leadwort (English), bleiwurz or zahnkraut (German), ensain (Arabia), sanza(Swahili), and inabiri (S- W Nigeria). It belongs to the Plumbaginaceae family that includes 10 genera and about 300 species.

Plumbago zeylanica, has been credited with therapeutic properties in treatment of various diseases like of diseases related to liver spleen, fever, dysentery, diarrhoea, leprosy. Apart from this it also helps in bacterial, microbial and helminth infections. The major portion of bioactive constituents like naphthoquinones, binaphthoquinones, coumarin and anthroquinones are present in the roots of the plant, so root is of utmost importance. In the form of paste external treatment is given for the treatment of skin diseases and leprosy.

Plumbagin an alkaloid present in leaves and roots of *Plumbago zeylanica* is a strong irritant but a powerful germicide. Plumbagin is a promising drug with various pharmacological properties that includes strong anticancer activity. Plumbagin has various effects Such as:

- In smaller doses stimulates muscular tissues.
- In larger doses paralyses muscular tissues.
- It stimulates the contraction of the heart and intestines muscular tissue.
- On the nervous system it shows stimulant action.

Figure 1*: Plumbago zeylenica*

Mostly by seed cultivation and semi-ripe cuttings *Plumbago zeylanica* is grown and propagated which are then preserved with growth regulators. As the sprouting of seed takes time and deterioration occur in germination rate by extended storage, traditional methods for propagation are challenging and less effective, also the content of secondary metabolite is very low. Hence, to increase the biomass and biochemical content of the plant minimize as well as growth time, in-vitro cultivation of this plant is the need of the hour.

In recent times, phytocompounds attained maximum interest to pharmaceutical industries for various therapeutic uses and drug designing (Chetri et al., 2016). However, of these bioactive molecules the synthesis is site-specific, depends on micro- and macronutrients availability and environmental conditions (Ramirez-Estrada et al., 2016). For the production of bioactive compound an alternative system has been employed i.e plant cell and organ cultures. (Ramirez-Estrada et al., 2016). And to enhance the productivity of bioactive componds several biotechnological approaches have been applied but for enhancing the production of desirable bioactive compound elicitation is known as the most practically feasible approach (Poulev et al., 2003; Angelova et al., 2006; Namdeo, 2007)

Small quantity of substance which applied induces the biosynthesis of specific compound are known as elicitor. “Elicitor plays an important role in the adaptations of plants to stressful conditions”. Elicitation is a method of enhanced production of bioactive compound to ensure plant survival in adverse conditions. To increase the biochemical and biomass content of plant, in-vitro shoot culture is an proficient strategy that can yield secondary metabolite at higher concentration.

For increasing bioactive substances of pharmaceutical interest shoot culture is an alternative system without depleting natural plant population.

This study examined the effect of abiotic elicitor on the production of plumbagin in *Plumbago zeylenica*. In-vitro shoot of *Plumbago zeylenica* were cultured on MS medium supplemented with various concentration and types of abiotic elicitor and the elicitor which are used are salicylic acid, sodium acetate and silver nitrate (50 µM). Shoot were investigated for plumbagin accumulation using HPLC. And phytochemical analysis was also done for elicited and non- elicited shoot culture.

LITERATURE REVIEW

From the past few years the dependency on synthetic medicine has got over and now the people are coming back towards the naturals with a hope of safety and security. Herbs are thus making a comeback as the only solution to dangerous and incapacitating effects of synthetic drugs. *Plumbago zeylanica* an important medicinal plant which is being used in the traditional system of medicines.

With a herbal 'renaissance' occurring across the globe, the demand of the plants are growing because of its wide rangle of biological acitivites. Plumbagin found in *Plumbago zeylanica* exhibit a wide spectrum of pharmaceutical activities such as anti-ulcer, anti-malarial, anti-obese, anti-microbial, anti-inflammatory, anticancer, antioxidant etc.

Morphology

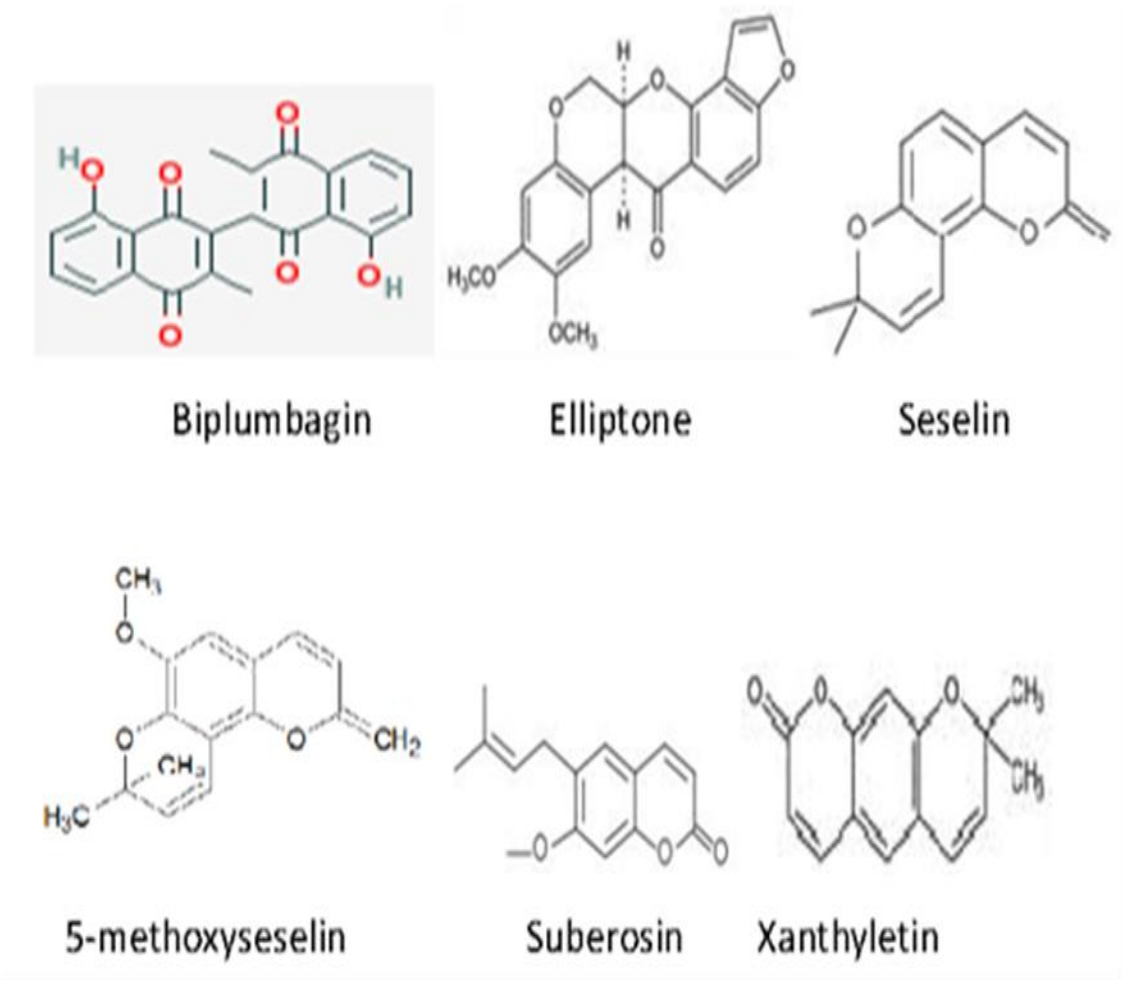
Still there is no proper classification observed from the previous literature whether *Plumbago zeylanica* is herb or shrub. Commonly it is considered as a perennial shrub but as observed in some of the literature it is herb. Generally, the plant is of height 0.5m to 2 m, having dark green 1.5 inch thick leaves and the intermittent distance between the leaves is approx 3 inch. The leaves are simple, alternate, elliptical or oblong, ovate, and they consume hairy margin. The petiole is thin and stipules are inattentive and with a 0-5 mm approx. tallness. Flowers are white in colour of diameter 0.5 to 0.75 inch with a stalk of size 4 to 12 inch.These exist in clusters. They are pentamerous, bisexual, regular, pedicellate and pleasant fragranced. The flowers have mucilaginous glands which help to trap the insects and insects help in pollination.

Calyx is dense and corolla is white in colour with tubular and slender. Stamens are 5 in number and free. Superior ovary, 5-gonous, one celled, ovule one, basal. Roots are light yellow in colour when it is fresh but changes to reddish brown when dried out. Roots often initiate in the form of hard pieces and the texture of the roots are unbroken and smooth and are usually very strong having distinctive odour with acrid and bitter taste.

Chemical composition

Herbal plant present on the earth contains various bioactive compounds which is very important due to its ability to act against a variety of diseases. These plants are an important asset of

pharmaceutical sector. Similarly *Plumbago zeylanica* being a medicinal herb also contains a variety of bioactive compounds which displays biological activity against various diseases. *Plumbago zeylanica* possess a variety of bioactive compounds like phenolic compounds, flavonoids, alkaloids, proteins, glycosides, steroids, saponins, triterpenoides, tannins, coumarins, carbohydrates, fixed oils, fats, sterols, and napthoquinones . Among all these "β-sitosterol" and "Plumbagin", are the most important bioactive compounds found in *Plumbago zeylanica*.



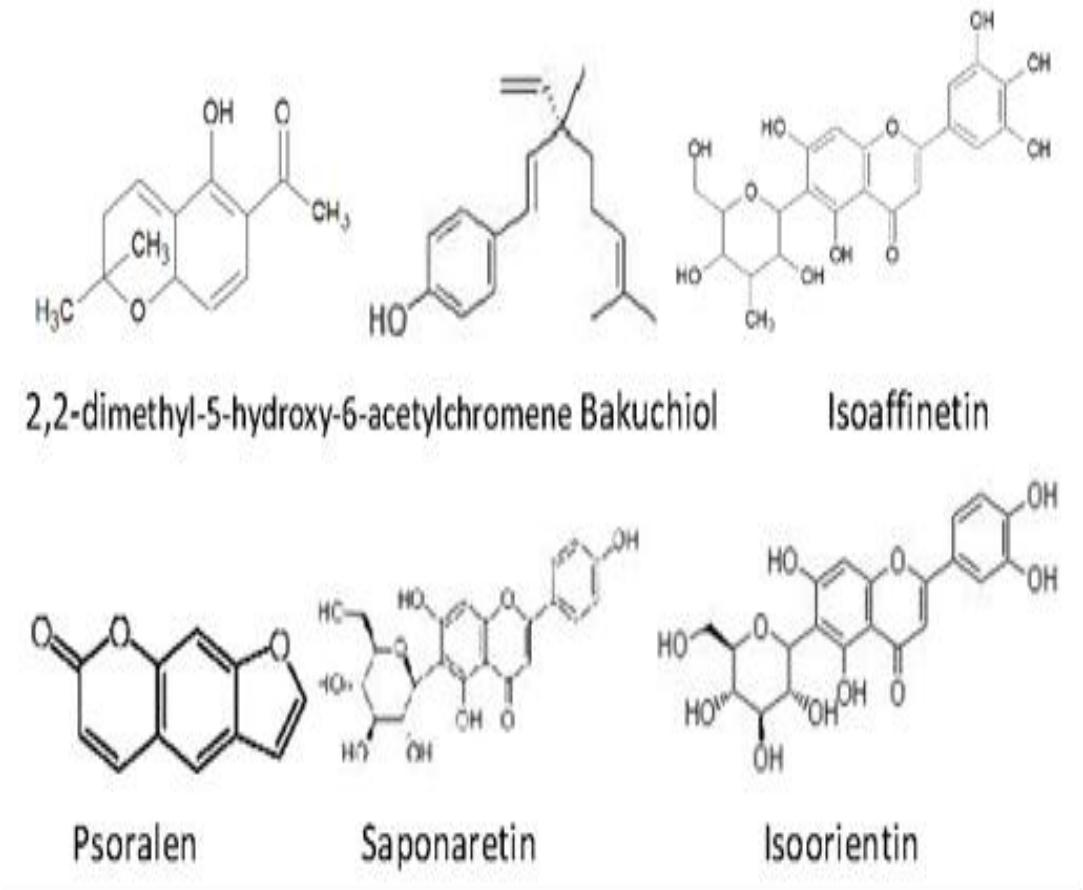


Figure 2: Chemical structures of some compounds present in Plumbago zeylanica

Propagation

Mostly by seeds and rooted shoots (bottom of plant*)* *Plumbago zeylanica* is grown that is available at the bottom of the plant, preserved with growth regulators. Usually the seeds sprout within 3 to 4 weeks but if the seeds stores for longer time then it leads to slower germination rate. Although the plant can be grown in various types of soil ranging from red to black soil but loamy (proper mixture of sand, silt and clay) to clayey soil with high organic content is most favoured. In natural habitats, the plants grow well in soggy soil with great organic content. They grow better in shady areas with moderately warm temperatures.

Traditional approaches is found to be problematic for completing the large scale demand at the commercial level. Also in traditional approach there is deprived seed propagation and ultimely

demise of seedlings on plantation below standard circumstances. To complete the high demand in-vitro proliferation is used. *Plumbago zeylanica* mass multiplication occur by means of leaf or root explants nodal explants, axillary buds and callus cultures.

Pharmacological activities:

Antimicrobial properties

Ahmed et al., (2007) examined alcoholic crude extract of *Plumbago Zeylanica* anti-bacterial property against the growth of *E. coli and Shigella* strain. Durga et al., (1990) found *E. coli* and *Staphylococcus aureus* delayed growth when inoculated in an antibiotic (streptomycin, rifampicin) but completely in the medium of plumbagin and antibiotic and thus regulate gastrointestinal flora (Iyengar and Pendse, 1966).

Aquil et al., (2006) evaluate anti- methicillin resistant *Staphylococcus aureus* (MRSA) presence in roots of *Plumbago zeylanica*. Inhibitory effect against bacteria and fungi is shown by *Plumbago Zeylanica* by paper disc method. Ethanol extract maximum activity has been seen against *Micrococcus luteus* (12mm). Significant antifungal activity was shown when compared with standard ethanol extract (Ravikumar and Sudha, 2011)

Anti-diabetic activity

For the obese patient with a low calorie diet 500mg of *Plumbago zeylanica* and 1gm of haridra powder in the form of capsules is given 4 times for 45 days, showed better results for weight loss in comparsion to Haridra when ethanolic extract at a concentration of 100mg. 200mg/kg along with tolbutamide was administered orally to the streptozotocin treated diabetic rats, reported decrease the activity of glucose-6-phosphate and meanwhile increasing the activity of hexokinase.

Anti- inflammatory activity

A study reported that anti-inflammatory activity is shown by hydro-alcoholic extract of *Plumbago zeylanica*. A study showed that reduction in oedema caused by *Plumbago zeylanica* thus comforting the body part, it is also examined to suppress the NF-kappa B activation in the tumour cells and also prevent graft versus host disease. A study revealed the anti-inflammatory influence of *Plumbago zeylanica* in carrageenin induced raw paw oedema in rats, the investigation was carried out in 4 groups, first two group was given 300mg/kg and they showed 31.03% acute

inflammation inhibition and the other two group was given 500mg/kg and they showed 60.30% acute inflammation inhibition. A clinical study conducted on 30 patients who were taken from the OPD and IPD of National Institute of Ayurveda, Jaipur by Napalchyal et al., where 4mgs of chitraka churna was given to 15 patient for twice a day with lukewarm water for 15 days. And they found a major improvement in the tenderness, pain, swelling, and dizziness that occur due to inflammation of the body parts.

Hypocholesterolemic activity

Sharma et al., (1991) carried out a clinical study in which serum cholesterol and LDL- Cholesterol reduced 53 to 86% and 61 to 91% respectively in the hyper-lipidemic rabbits by using an active component plumbagin. Accumulation of cholesterol and triglycerides prevented by plumbagin in liver and aorta.

Another study revealed the reduction of the cholesterol, serum cholesterol, LDL, and triglycerides on administration of 500mg/kg of ethanolic extract of *Plumbago zeylanica* root for 60 days with normal diet to hyper-lipidaemic rabbits and when it is given with Vitamin E and triglyceride then total cholesterol and LDL cholesterol was significantly reduced (Ram, 1996).

Hepatoprotective

Increased hepatic hexokinase activity and decreased hepatic glucose-6- phosphatse, alkaline phosphalase (ALP), serum acid phosphatase (ACP), and lactate dehydrogenase (LDH) was shown in streptozotocin diabetic rats on oral administration of ethanolic extract (100-200mg/kg) for 6 weeks. Improvement in biochemical damages states it's hypoglycaemic and hepato-protective nature in streptozotocin induced diabetic rats (Zarmouh et al., 2010).

Wound healing activity

Jyothi et al., and Kodati et al., (2013) found wound healing activity in ethanolic root extract of *Plumbagin zeylanica* of Wistar rats. Phytochemical presence in root extract of *Plumbago zeylanica* is responsible for wound healing activity. Other studies (Bryan et al., 2012; Schremi et al., 2010; Kumar et al., 2015) provided evidence for pathogenesis of non-healing ulcers for a role of oxidative stress. Wound healing normal physiology depends on low levels of oxidative stress and reactive oxygen species, impaired wound healing caused by overexposure to oxidative stress.

Wound oxidative stress controlled by antioxidants and thus accelerate wound healing (Jyothi et al., 2013)

Cytotoxicity

Cellular proliferation, radio-resistance and carcinogenesis modulated by plumbagin present in *Plumbago zeylanica*. By the activation NF- Kappa B activation pathway of transcription factor all these reactions are regulated. Constitutive NF- Kappa B activation present in certain tumour cells suppressed by plumbagin, over all. The authors believe that potent inhibitor is plumbagin of NF Kappa B activation pathway, gene products suppression occur.

Toxicity Study

Plumbagin an active compound of *Plumbago zeylenica* may have potential as a compound in synthetic insecticides. When taken orally or applied roots are reported to be powerful poison ostium uteri, causing abortion. The methanol root extract of *plumbago zeylenica* when given to rabbits showed limilted toxic effects and did not produce any overt signs of toxicity in skin.

Bioactive Compound – Plumbagin

Plumbagin is a bioactive phytoconstituent and it is yellow crystalline (Navneet et al, 2010). It is a naphthaquinone which is natural and it shows a broad range of pharmaceutical properties (Arunachalam et al., 2010).

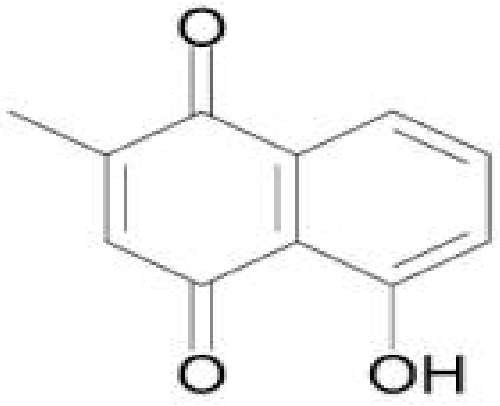


Figure 3: Plumbagin

Plumbagin

Plumbagin shows to have anti-carcinogenic activity (Eldhose et al., 2014) antifungal, cardio-protective, anti- malarial, hyperglycemic, anti-inflammatory, anti-atherosclerotic. (Yuan-Chuen et al., 2005; Yen-Ju et al., 2006; Vanisree et al., 2004). It also shows anticancer properties (Singh and Udupa, 1997)) and studies on embryonic fibroblast cells of mouse which suggest that the plumbagin cytotoxic action may be because of apoptotic species cascade by the generation of reactive oxygen species (ROS) (Srinivas et al., 2004) such as hydrogen peroxide and superoxide anion.(Kawiak et al., 2007). This might accounts for its apoptotic effects and cytotoxic (Su-Jung et al., 2010; Ganeshan and Gani, 2013).

In-vitro studies in Plumbago zeylanica

Growth and propagation of *Plumbago zeylanica* occur mostly by seed cultivation, semi-ripe cuttings that are preserved with growth regulators. Growth of *plumbago zeylanica* occur mostly by seeds also by rooted shoots (bottom of plant) or by rooted shoots that is available at the bottom of the plant or by semi-ripe cuttings, preserved with growth regulators. Usually within 3 to 4 weeks the seeds sprout but if the seeds stores for a longer time then germination rate slows down. Favoured method of *Plumbago zeylanica* plants proliferation is propagating seeds in a nursery with consequent transplantation addicted to the ground at a density of 58 x 58cm.

A study reported MS media supplemented with 27.2μM Adenine Sulphate + 2.46 μM IBA and rooting in MS media supplemented with 4.92 μM IBA shows maximum shooting. Dohare et al., observed maximum number of roots in half strength MS media supplemented with 1mg/l IAA and maximum number of shoots in MS media supplemented with 1mg/l BA+ 1mg/l NAA. A report shows MS media containing 0.1 mg/l NAA+ 1.5 mg/l Kinetin from nodal explants gives the maximum number of roots.

MATERIAL AND METHOD

Plant collection

Eight different accessions (539867, 256070, 439214, 524440, 524441, 421418, 398891 and 6024281) of *Plumbago zeylanica* were obtained from National Bureau of Plant Genetic Resource (NBPGR), New Delhi. These accessions were maintained in MS media at plant tissue culture laboratory of Department of Biotechnology, Delhi Technological University.

Preparation of MS medium added with growth regulator

MS medium was prepared by mixing all the constituents i.e .,macronutrients($CaCl_2.2H_2O$,NH_4NO_3,KNO_3,KH_2PO_4,$MgSO_4.7H_2O$,),micronutrients($ZnSO_4.4H_2O$(H_3BO_3,$MnSO_4.4H_2O$,KI,$Na_2MoO_4.2H_2O$,$CoCl_2.6H_2O$,$CuSO_4.5H_2O$,$FeSO_4.7H_2O$,Na_2EDTA) ,vitamins(niacin, pyridoxine, thiamine,glycine,),M-inositol,carbon source(sucrose)in 500ml distilled water and then pH was adjusted to 5.8 by using 1N HCl. After that the final volume was made up to 1000ml with distilled water. Then 0.8% solidifying agent agar was added and the media was sterilized by autoclaving it at 15psi or 121°C for 15 minutes. Then the medium was cooled at room temperature before adding some hormone (200µl BAP) and lastly pouring was done in flasks and culture tubes.

Elicitor preparation

Stock solution of sodium acetate, silver nitrate, salicylic acid were prepared. Sodium acetate and silver nitrate (50 µM) were prepared in distilled water. Salicylic acid (50µM) was prepared in ethanol. Salicylic acid, sodium nitrate and sodium nitrate were filter sterilized using syringe filter and then added to culture medium.

Treatment with elicitor

Nodal stem of *Plumbago zeylanica* were used as explants. Inoculation of explant were done onto fresh MS medium containing 200µL BAP supplemented with salicyclic acid, silver nitrate and sodium acetate (50µM).These were added to the individual flasks aseptically. Flask without any elicitor were kept as control. The lid of the culture tube was then closed carefully and sealed with parafilm. The culture tubes were labelled accordingly and then incubated at 25±2°C temperature in a culture room with a photoperiod of 8 hour dark, 16 hours light, and 65 humidity.

Plumbagin quantification

Plumbagin was quantified by using HPLC. Methanol and water in the ratio of 80:20 was used as mobile phase in C18 column with a flow rate of 1.0ml/min. Wavelength of 272nm was detected using UV detector. Plumbagin calibration curve was plotted using standard plumbagin over concentration range 100-500µg/ml.

Phytochemical analysis

Chemical tests were performed on different accessions of elicited and non-elicited in vitro cultured of *Plumbago zeylanica* for the estimation of total phenolic content, total tannin content and antioxidant potential.

Preparation of plant extracts

1g of plant material was measured and was finely grounded using mortar and pestle. One gram of this grounded plant material was soaked in 10 ml methanol and placed in a shaker device for 1-2 minutes at room temperature, and then stored in a refrigerator for 2 days. After that, organic fraction was filtered by using syringe filter and was stored at 4°C in the refrigerator for further use.

Quantitative analysis of phytochemical constituents.

Total phenol

The quantity of phenols is determined using the spectrophotometer method. 200µl of plant extract was taken into test tube, and 1.5ml of Folin-Ciocalteu reagent is added. After the addition of Folin-Ciocalteu reagent, the mixture was kept in dark for 5 minutes. After that, 1.5ml of 5% sodium carbonate was added to the mixture. The sample is made up to the mark and left for 2 hours to react for colour development and measured at 750 nm wavelength using a spectrophotometer.

Total tannin

Quantity of tannins is determined by using the spectrophotometer method. 100µl of plant extract was taken into test tube, and 7.5ml of distilled water is added. After the addition of distilled water, 0.5ml of Folin-Ciocalteu reagent and 1ml of 35% sodium carbonate is added to the mixture. Shake the constituents of the test tube and left for 30 minutes at room temperature to react for colour development and measured at 700nm wavelength using a spectrophotometer.

Analysis of antioxidant activity

Antioxidant activity of leave extracts was determined by using the DPPH method.100 µl of extract was mixed with 3.9 ml of dpph solution. The mixture was vortexes and incubated for 30 min. The optical densities of the solutions were measured at 517 nm using spectrophotometer

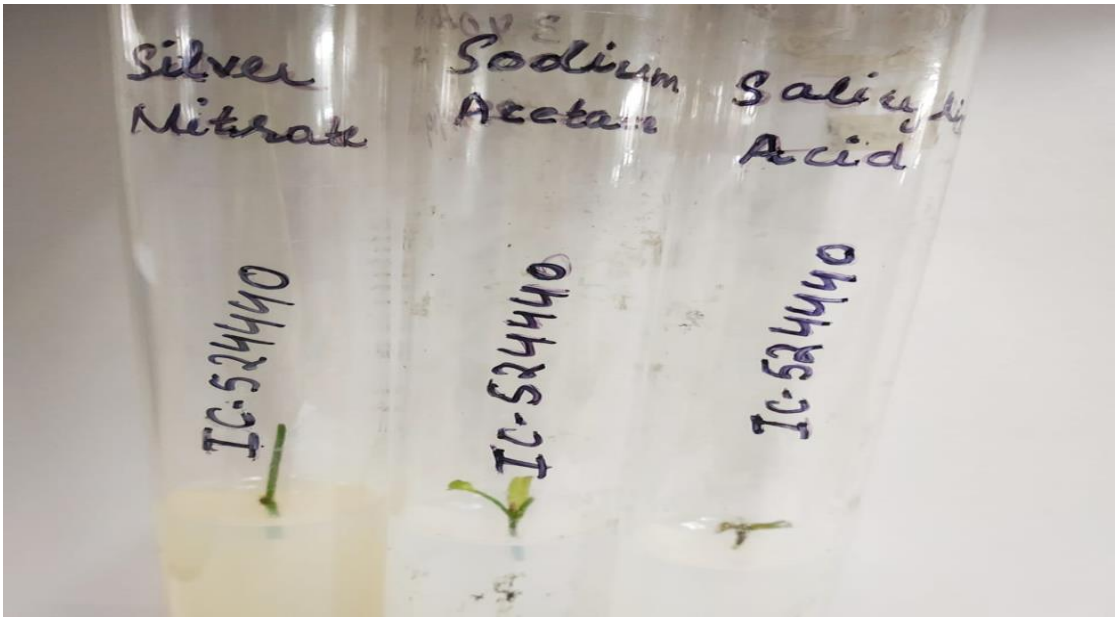


Figure 3: In-vitro culture of *Plumbago zeylanica* in MS medium with elicitor(IC-524440)

Figure 4: In-vitro culture of *Plumbago zeylanica* in MS medium with elicitor(IC-539867)

Figure 5: In-vitro culture of *plumbago zeylanica* in MS medium with elicitor(IC-421418)

Figure 6:In-vitro culture of *plumbago zeylanica* in MS medium with elicitor(IC-524441)

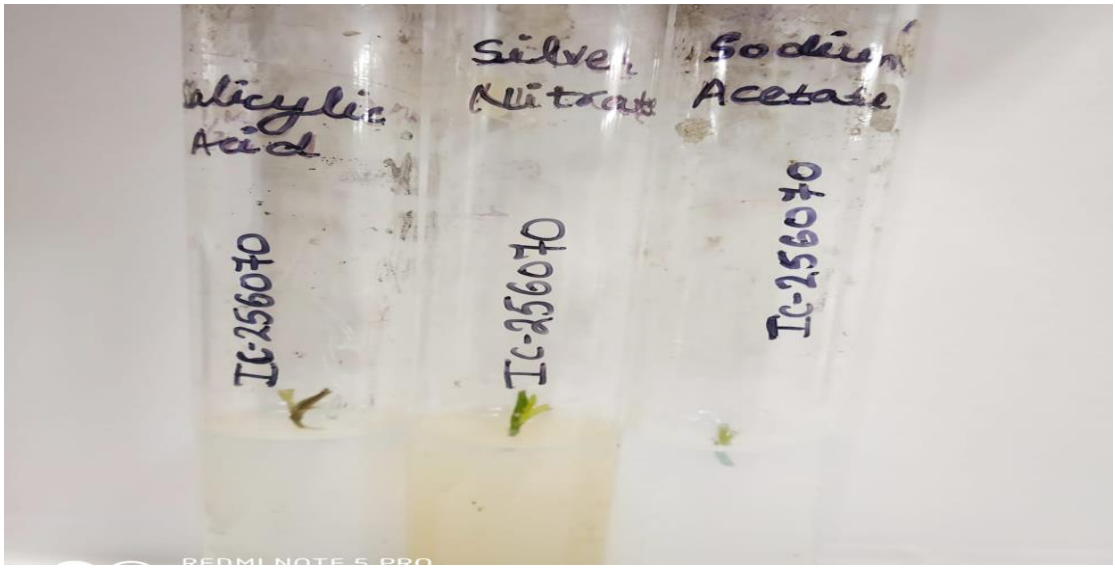


Figure 7: In-vitro culture of *plumbago zeylanica* in MS medium with elicitor (IC-256070)

Figure 8: In-vitro culture of *plumbago zeylanica* in MS medium with elicitor (IC-439214)

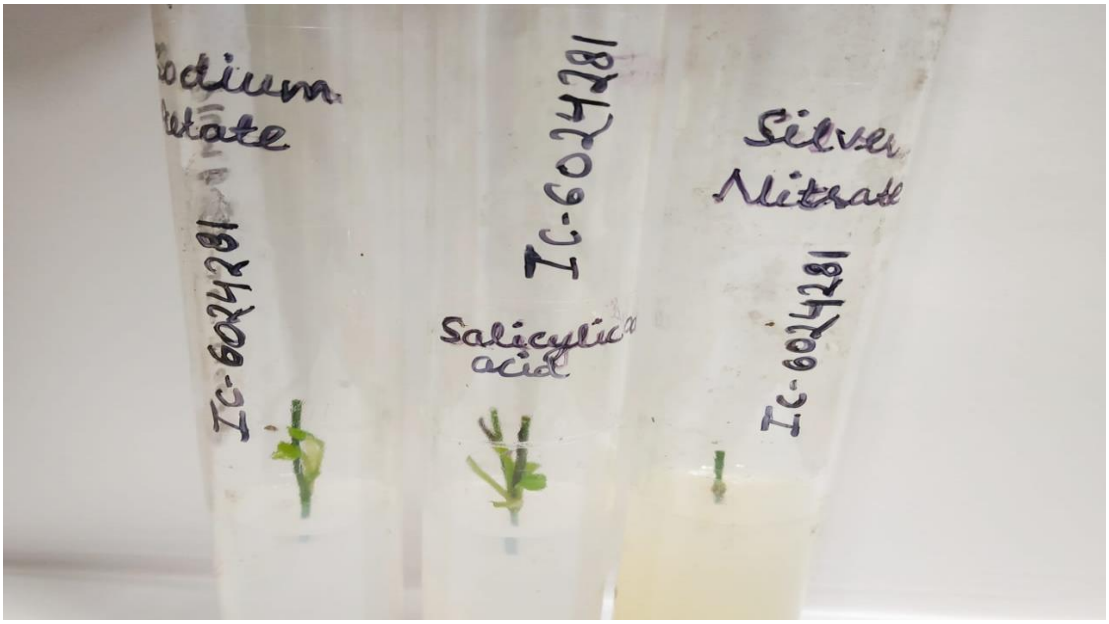


Figure 9: In-vitro culture of *plumbago zeylanica* in MS medium with elicitor(IC-6024281)

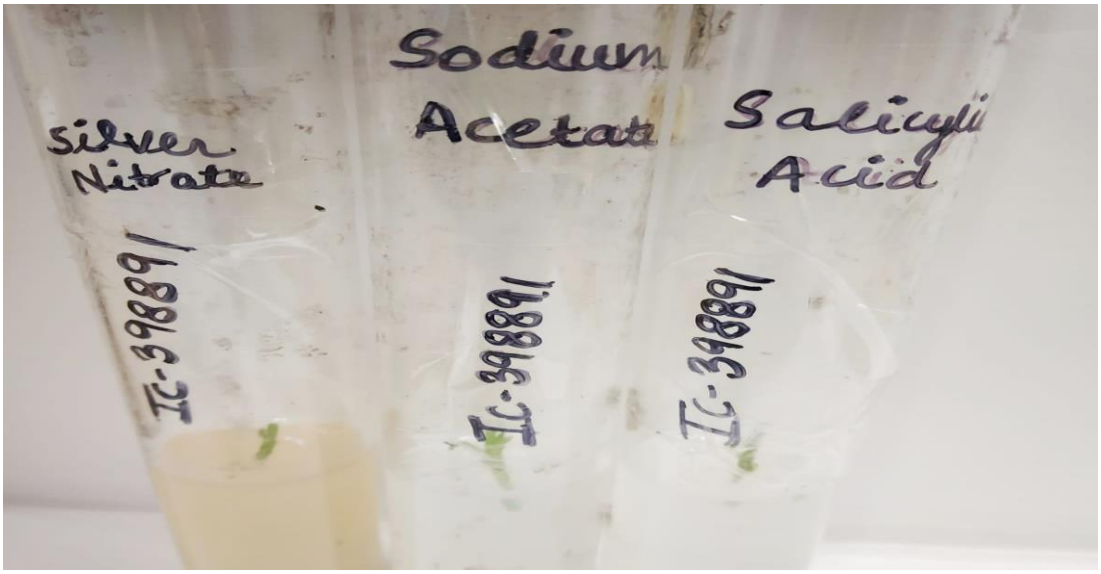


Figure 10: In-vitro culture of *plumbago zeylanica* in MS medium with elicitor(IC-398891)

RESULT AND DISCUSSION

For secondary metabolite production modern method are currently being used that are improved by biotechnological which includes organizing nutrient arrangement, modifying environmental conditions and applying specialized technique as immobilization, permeabilizatin, hairy root culture, precursor feeding and especially elicitation to increase secondary metabolite production. Elicitaion is a phenomenon in which plant cell responds to treatment to produce the desired secondary metabolite by using abiotic and biotic elicitor. From microbial extract biotic elicitor are generally prepared while chemical and physical factor that enhance secondary metabolite production in plants refer as abiotic elicitor. Naik and Al-Khayari (2016) reported that abiotic elicitor enhances bioactive production in several plants. To enhance secondary metabolite production using the optimal type and concentration of elicitor are crucial. In our study, effect of elicitor on plumbagin production from in-vitro shoot cuture were observed. Cultures were pretreated with types and concentration of abiotic elicitor (Salicylic acid, sodium acetate and silver nitrate) and then harvested for plumbagin analysis.

Effect of Salicyclic acid on plumbagin production Salicylic acid (SA) is the main signals in plants for defence gene expressions that regulate pathogens resistance also elicit production of bioactive compounds in plants. Using 50µM of salicylic acid, the shoot treated produced plumbagin content is expected to be higher than the control which indicated that plumbagin mainly accumulated in the intracellular levels.

Effect of silver nitrate on plumbagin production

In *plumbago zeylenica* silver nitrate greatly affect production of plumbagin. To stimulate production of secondary metabolite metal ions such as Ag have been used. Successful published research showed secondary metabolite elicitation in *Brugmansia candida* using silver nitrate and tanshinone production in *Perovskia abrotanoides.* Using 50µM silver nitrate as an elicitor plumbagin production will be higher than the control.

Effect of sodium acetate on plumbagin production

For obtaining higher concentration of product for commercialization, various efforts have been made to stimulate the biosynthetic activities of cultured cell by using several methods. Here, the

content of plumbagin was enhanced by the addition of sodium acetate. Using 50µM sodium acetate plumbagin production will be higher than the control in *Plumbago zeylanica*.

Phytochemical studies

Bioactive compounds that are present in plants are formed during metabolic process are Phytochemicals. The presence of plant constituents are reveal by these compound therefore for pharmaceutical applications of plants estimation of these compounds is required. By elicitation of in-vitro cultures it can also be enhanced. In this study total phenol, tannin content and antioxidant activity of elicited and non-elicited *plumbago zeylanica* shoot culture were estimated to access the medicinal importance of this plant. The antioxidant activity was determined by using the DPPH method. Antioxidant activity of elicitor and non-elicited shoot culture was evaluated. Antioxidant activity of silver nitrate, salicylic acid and sodium acetate elicited shoot culture is expected to be higher than non-elicited culture. Total phenolic content and total tanin content of silver nitrate, salicylic acid and sodium acetate elicited shoot culture will be higher in case of elicitor treated shoot cultures. This may be due to improved production of total polyphenolic compounds in the elicitor treated shoot culture.

CONCLUSION

Plumbagin a bioactive compound present in *plumbago zeylanica* is used for various diseases treatment like of diseases related to liver spleen, fever, dysentery, diarrhoea, leprosy. In this study abiotic elicitor (Salicylic acid, sodium acetate and silver nitrate) affect the accumulation of plumbagin in *Plumbago zeylanica*. Phytochemical analysis was also done for elicited and non-elicited shoot culture. Result indicate that by using elicitor increase in plumbagin production as well as phytochemical occur in shoot culture of *Plumbago zeylanica*.

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