Estimation of plumbagin by RP-HPLC & FAME analysis by GC-MS of important medicinal plant *Plumbago zeylanica*

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 "***Estimation of plumbagin by RP-HPLC & FAME analysis by GC-MS of important medicinal plant Plumbago zeylanica"*** **(2K14/IBT/10)** in the partial fulfillment of the requirements for the reward of the degree of M. Tech, 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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DECLARATION

This is to certify that the thesis of Major Project entitled "**Estimation of plumbagin by RP-HPLC & FAME analysis by GC-MS of important medicinal plant *Plumbago zeylanica***" 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 my guidance of the 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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2K15/IBT/10.

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Estimation of plumbagin by RP-HPLC & FAME analysis by GC-MS of important medicinal plant *Plumbago zeylanica*

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ABSTRACT

Plumbago zeylanica is an important medicinal plant used in the cure of various diseases. Due to its various therapeutic values, this plant is being researched for high biomass production as well as achievement of maximum yield of bioactive compounds. In the present study, effect of different media and carbon source on *in vitro* shoot regeneration of five different accessions of *Plumbago zeylanica* using nodal explants was investigated. Among the three types of carbon sources that were employed in the present study, sucrose proved to be a better choice for multiple shoot regeneration followed by fructose and glucose in five different accessions of *P. zeylanica*. MS medium provided maximum growth among four different media tested i.e. MS, White, B5 and Nitsch. Quantitative analysis of important bio-active compound plumbagin was performed using standard protocol by reverse phase high performance liquid chromatography of methanolic extract of roots of accession number 524441 which was found a potential accession in a previous study of plant elicitors. Elicitors enhanced plumbagin concentration three times in comparison with the reference. In addition to these three studies, shoots of this plant was also used for fatty acid methyl ester (FAME) profiling. FAME analysis concluded that this plant is rich in C14, C15, C16 and C18 fatty acids. Maximum percentage of fatty acids is found in accession number 421418. Accession number 524441 showed presence of each fatty acids.

INTRODUCTION

Since Vedic age, plants have been a source for herbal medicines. Detailed descriptions of plant and plants products about 700 herbs used for medicinal purposes to cure various ailments have been mentioned in various literatures like *Ayurveda*, *Charak samhita* and *Susrut Samhita.*

Plumbago zeylanica (Chitrak; Plumbignaceae) is a medicinal plant having multipurpose medicinal property and is most commonly used in traditional medicinal system of India. This plant being a native of South Asia, the species is widely distributed among tropics and subtropics growing on an altitude of up to 2000m from sea level of deciduous woodlands, savannas' and scrublands (Sharma A *et al*., 2015). The plant is commonly known as Leadwort, Chitrak etc. which comprises of 10 genera and 280 species. Among which *P. zeylanica* is widely cultivated for having highest therapeutic value.

As all the herbal plants contain some amount of bioactive and therapeutic compound so does *P. zeylanica.* Imperative compounds are present in this plant which displays a variety of action. Secondary metabolites like alkaloids, flavonoids, napthoquinones, glycoside, saponins, steroids, triterpenoids, coumarins, phenolic compounds, tanins, carbohydrate, fixed oils, fats and proteins are present in different parts of the plant. Among all these bio-active compounds, plumbagin is the most important principle active compound. Therapeutic uses according to various pharmacological studies includes anti-microbial, anti-fungal, anti-inflammatory, anti-hyperglycemic, anti-cancer, anti-plasmodial, anti-atherosclerotic activity etc.

P. zeylanica is grown and propagated mostly by seed cultivation, semi-ripe cuttings which are preserved with growth regulators. Due to the long time sprouting of seed in 21-30 days and the deterioration in germination rate by extended storage, traditional approaches for proliferation are problematic and less efficient, also the secondary metabolite content is very low. Hence, to minimize the growth time as well as to enhance the biomass and biochemical content of the plant, in-vitro cultivation of this plant is the need of the hour.

Previous studies reported that carbon source is essential for the growth of the plant and they also have important role in metabolic pathways (Panathula *et al.,* 2014) to enhance the formation of auxiliary buds and branching of adventitious roots (Saad *et al.,* 2012). Plant cells in vitro, shows physiological and morphological responses to microbial, physical or chemical factors which are known as 'elicitors'. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants to ensure their survival.

OBJECTIVES:

1. **In-vitro Propagation:** Effect of different media (MS, White, Nitsch, B5) and various carbon sources (sucrose, glucose, and fructose) on growth and shoot proliferation on five different accessions of *P. zeylanica* was tested.

2. **Gas Chromatography Analysis**: Fatty acid methyl ester (FAME) analysis in five different accessions of *P. zeylanica* by GC-MS to identify their profile of fatty acids.

3. **Reverse Phase High Performance Liquid Chromatography (HPLC):** Quantification of plumbagin using RP-HPLC technique for the accession which was found best in the cultures of yeast extract.

REVIEW OF LITERATURE

Plumbago zeylanica commonly known as Chitrak or lead wort is a white flowered plant which is native to South Asia. It is widely dispersed in tropics and subtropics of the world and budding in savannahs, deciduous woodland, scrublands from sea level up to altitude of 2000 m [Vijver 2013, Aditi G., 1999]. In India it is spotted in central India to West Bengal, Maharashtra, and Uttar Pradesh and also to some parts of South India. Most commonly used name persisted with the plant is chitraka [mandavkar and jalalpure, 2011]. Plumbago is from Plumbaginaceae family comprising of 280 species and 10 genera. The genus Plumbago takes into account 3 species *Plumbago indica L. (P. rosea L.) P. capensis L., and P. zeylanica L.,* among these *Plumbago zeylanica* having most therapeutic usage is highly cultivated plant. It is one of the oldest herb which is reported to be used in Ayurveda for several disorders over thousands of years. The plant grows wild in India and is now-a-days also refined commercially.

Morphology

The previous literature reported shows no uniformity stating whether *P. zeylanica* is a shrub or herb. While it is a perennial bushy shrub some of the works has defined it as herb while some work has given the plant class of shrub. Leaves of *P. zeylanica* are dark green in Color and are simple, elliptical with hairy margins along with alternate placement on the stem with the distance of up to 3 inches and thickness of 1.5inch. The petiole is thin and with an approximate length of 0.5 mm and native stipules are present. The plants breed flower white in color with diameter of 1/2 to 3/4 inch having the stalk measuring 4 to 12 inches along with a terminal raceme-type of inflorescence. Flower of P. zeylanica is pentamerous, regular, bisexual with a pleasant fragrance existing in a cluster or bunches. The pollination of these plant is brought by insects with flowers coming round the year, the flower possess a gland secreting a mucilaginous substance which helps the plant intraping the insect on it to carry the anthers form one flower to other. Corolla of the flower is tubular and slender in shape have white color while the calyx is enclosed with stalked and is densely arranged, various gland are present secreting mucous like

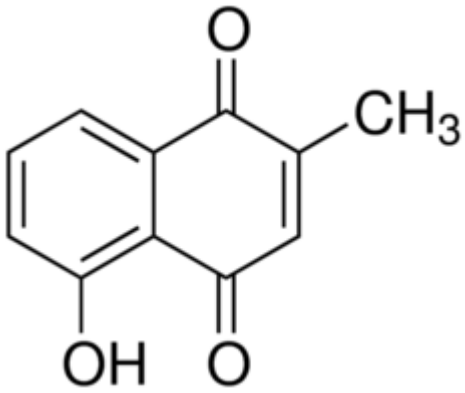
substance thus making the flower sticky. The flower possess a basal, single ovule, pentagonous and superior ovary.

Roots of *P. zeylanica* are long and slightly branched with very less secondary roots, having a smooth and unbroken texture, color of the roots is light yellow when the plant is freshly plucked out of the ground and changes to reddish brown in color when it is dried which often initiates in the form of hard pieces. These roots are usually very strong having a bitter taste and a distinct odour with acrid.

Chemical composition

Every herbal plant present on the earth consists of various bioactive compounds which is very important as these bioactive compounds have the ability to act against a variety of disease. These plants are an important asset for today's pharmaceutical sector. Similarly *Plumbago zeylanica* being a medicinal herb also contains a variety of therapeutic or bioactive compounds which displays an imperative activity against various diseases. *P. zeylanica* possess a variety of secondary metabolites like flavonoids, alkaloids, glycosides, steroids, saponins, triterpenoides, tannins, coumarins, phenolic compounds, carbohydrates, fixed oils, fats, proteins, sterols, and napthoquinones . Among all these "**β-sitosterol**" and "**Plumbagin**", are the most important bioactive or therapeutic compounds present in *P. zeylanica.*

Plumbagin



Plumbagin (5-hydroxy-2-methyl-1, 4- naphthoquinone- C_{11}-H_8-O_3) is a napthoquinones which is mostly present in roots of the plants which is a stirring yellow pigment that patently appear in the Plumbaginaceae family members and is 1% of the entire plant . The leaves contains no Plumbagin while a very little amount is present in the stem of *P. zeylanica* and because of the presence of napthoquinone Plumbagin is a natural yellow pigment which exists in the form of needles. Plumbagin is readily soluble in organic solvents like acetone, chloroform, alcohol, benzene and acetic acid also this compound is exceedingly corrosive with toxic possessions. The therapeutic properties of Plumbagin includes antimicrobial activity, reports state that methanolic extract depicts strong activity against *Escherichia coli, Salmonella typhi and Staphylococcus aureus* while the aqueous extract is found to have less antibacterial action. Plumbagin also shows anticancer , antifungal. Similar to that of colchicine, a very small amount of Plumbagin displays antimitotic progression.

Propagation

Naturally *P. zeylanica* grows mostly by seeds which is utilized by various cultivators involving traditional methods and also using rooted shoots which are available at the plant bottom. Semi-ripe cutting that are preserved using growth regulators are also taken for growing of plant. Stored seeds or seed sprout with extended storage of over 3months or 21—30 days respectively shows severe descent in germination rate. The most favoured method for proliferation is propagation of seeds in a nursery followed by subsequent transplantation. The plant naturally grows well in soil with high organic content and moisture with in-between warm temperatures and place having moderate shade. Traditional proliferation approaches have been mostly problematic and nonsufficient in encountering escalating demands of the plant for commercial pharmaceutical sector. To cope with the mounting request the *in vitro* proliferation method is the most effective tool.

Pharmacological Activities

P. zeylanica has been reported to possess a variety of pharmacological activities like anti-inflammatory, wound healing, anti-diabetic, memory inducing, lipid metabolism, anti-malarial, allergic and modulatory, anti-fertility, anti-bacterial, anti-viral, anti-cancer, anti-oxidant, larvicidial. The roots are used as a traditional medicine in various regions like in Ethiopia the root, bark or leaf powder is used for treatment of syphilis, tuberculosis and gonorrhoea and in Zambia the root and leaf is used as a remedy for inflammation of mouth, chest and throat by boiling the plant part in milk and consuming it.

Plumbagin which is isolated from roots of *P. zeylanica* was reported to significantly reduce the serum cholesterol by 53-86% and LDL- cholesterol by 61-91% when administered to the hyperlipidaemic rabbit model. It has also been reported that Plumbagin isolated from *P. zeylanica* has a significant increase on GLUT4 translocation in case of Streptozotocin induces diabetic rats. [Christudas Sunil and *et al.2012*]. Studies done on effect of *P. zeylanica* on Central Nervous System reveals that 50% ethanol extract of root showed significant increase in spontaneous motility in rats. The study shows that the Plumbagin from the roots of *P. zeylanica* enhances the anti-bactericidal activity of peritoneal macrophages of BALBS mice against *staphylococcus aureus* at lower concentration but inhibits the activity at higher concentration [Abdul and Rachender, 1995].

Various reports state that the plant *P. zeylanica* consists of bioactive compound which possess anti-cancer activity against various cancer cell lines. Study on anti-cancer activity reveals that ethanolic extract of *P. zeylanica* possess significant anti-cancer activity against Ehrlich Ascites Carcinoma in animal model, and also it reduces elevated level of lipid peroxidation having presence of higher terpenoids and flavonoids [Hiradeve *et al., 2009].* Studies also reveals that Plumbagin can inhibit cell proliferation, block cell cycle and induce apoptosis of APL cell line NB4 cells [Zhao and Lu, 2006].

MATERIAL AND METHODS

1- TISSUE CULTURE

Materials

Five different accessions (398891, 524441, 421418, 439212, and 539866) of *P. zeylanica* were obtained from National Bureau of Plant Genetic Resource (NBPGR), New Delhi which were previously cultured in-vitro. From these, nodes and internodes were taken as explant to further continue its micro propagation and afterwards these plantlets were used for different experimental conditions.

Experimental conditions

In this study the plants were taken under aseptic condition in a laminar airflow chamber and were transferred to different culture tubes with varying conditions.

1) Role of different media on shoot proliferation of *P. zeylanica*

The explants form each accession (398891, 524441, 421418, 439212, and 539866) were taken and inoculated on different media under aseptic condition inside a laminar airflow chamber. Different media used for inoculation are Murashige and Skoog (MS) 1962 with sucrose3% w/v and 0.8% w/v agar, Gamborg's B5 media (HiMedia Laboratories Pvt. Ltd., India), White Media (HiMedia Laboratories Pvt.Ltd, India), Nistch Media (HiMedia Laboratories Pvt. Ltd.), these media were supplemented with 1mg/ml of Benzyl Amino Purine as a plant growth hormone and the pH of the media was maintained at 5.8.

The media prepared was autoclaved at a temperature of 121°C and a pressure of 15psi for 20min. The media was then cooled to hand bearable temperature and the growth hormone was mixed, after which media was poured in the culture tubes. These tubes were left overnight to solidify for inoculation of the explant. The explants were inoculated under aseptic environment and incubated for a photoperiod of 16h at 25±2°C for 8 weeks.

2) Effect of different carbon source on shoot proliferation of *P. zeylanica*

MS media was taken and the carbon source in the media was replaced by glucose and fructose. Then the explants were inoculated in the media inside a laminar airflow chamber under aseptic condition. Then explants were incubated at 25±2°C for 8weeks with a photoperiod of 16h.

Data analysis: Observations were recorded and are presented as means ± standard deviation of 3 biological replicates to estimate the variability between the five accessions.

2- GC-MS Analysis of Fatty Acids of *P. zeylanica*

Materials-

100 mg leaves of each accession, mortar pestle, screw cap glass tubes, 2% methanolic HCl, NaCl solution, hexane, centrifuge, liquid nitrogen, GC-MS setup.

Method

Preparation of FAMEs for the GC-MS analysis of fatty acids of *Plumbago zeylanica.*

- 100mg of leaves of each accession of *Plumbago zeylanica* were slightly crushed in a mortar pestle and then transferred to a screw-cap glass tubes.

- Added 1 ml of 2% methanolic HCl to the crushed leaves. The samples were then incubated at 90°C for an hour.

- After that 0.9% of NaCl in H_2O was added followed by 2 ml of hexane and mixed.

- The samples were then centrifuged for phase separation at 2000 rpm for 2 minutes.

- After centrifugation, the upper (hexane) layer of the sample was transferred into a fresh glass tube and dried under nitrogen flow.

- Dried samples were then diluted with 100µl of hexane. The samples (1µl) were then injected into GC-MS for analysis one by one.

- Chromatogram was obtained and results were analyzed.

3-Plumbagin quantification by RP-HPLC

Five accessions (398891, 524441, 421418, 439212, 539866) were culture on MS media supplemented with four different elicitors i.e., Jasmonic acid, Salicylic acid, Yeast extract and Chitosan. The explants were inoculated on the media and were incubated at 25±2°C with a photoperiod of 16h for 8weeks and growth was observed. The accession with maximum growth in terms of number of shoots and number of nodes was taken for the Plumbagin quantification.

Equipment of HPLC

HPLC system including: a pump, an injection port, column compartment, and UV- VIS detector. Fused core C18 HPLC column, syringe filter, 0.22 micrometer.

Standard preparation

Weigh 5 mg of plumbagin standard and dissolve in 15ml of methanol.

Preparation of sample for estimation of plumbagin using HPLC

- Weight 150mg of air dried powdered root sample into the glass tube.
- Add 10 ml of methanol and keep it for 24hrs for cold maceration in -4°C.
- After cold maceration filter the sample with 0.45 micrometer filter paper.
- Filter a portion of solution through a 0.2 micrometer polytetrafluoroethylene (PTFE) syringe filter and inject into HPLC.

Chromatographic conditions

Column Fused core C18.

Mobile Phase	Water: Methanol (10:90)
Flow rate	1ml/min
Inj Volume	15 microliter
Column Temp.	260°C
Detection	UV at 268nm.

Procedure for HPLC

HPLC system was calibrated with the mobile phase before injecting the samples until a stable base line was obtained. It took approx. 20 minutes for calibration. After this, 15ul standard solution was injected into the column and was allowed to rum for 15 minutes. On completion of the run the column is washed so as to remove any kind of impurities that can affect the run of other samples. Once the column is washed properly basal sample solution which contained only MS + BAP i.e. no elicitor was injected and run. Similarly samples containing elicitor along with MS + BAP were injected and run. These samples were prepared in triplets. Each sample took 15-20 minutes to run. Chromatograms were obtained for each and were further analyzed.

RESULTS AND DISCUSSION

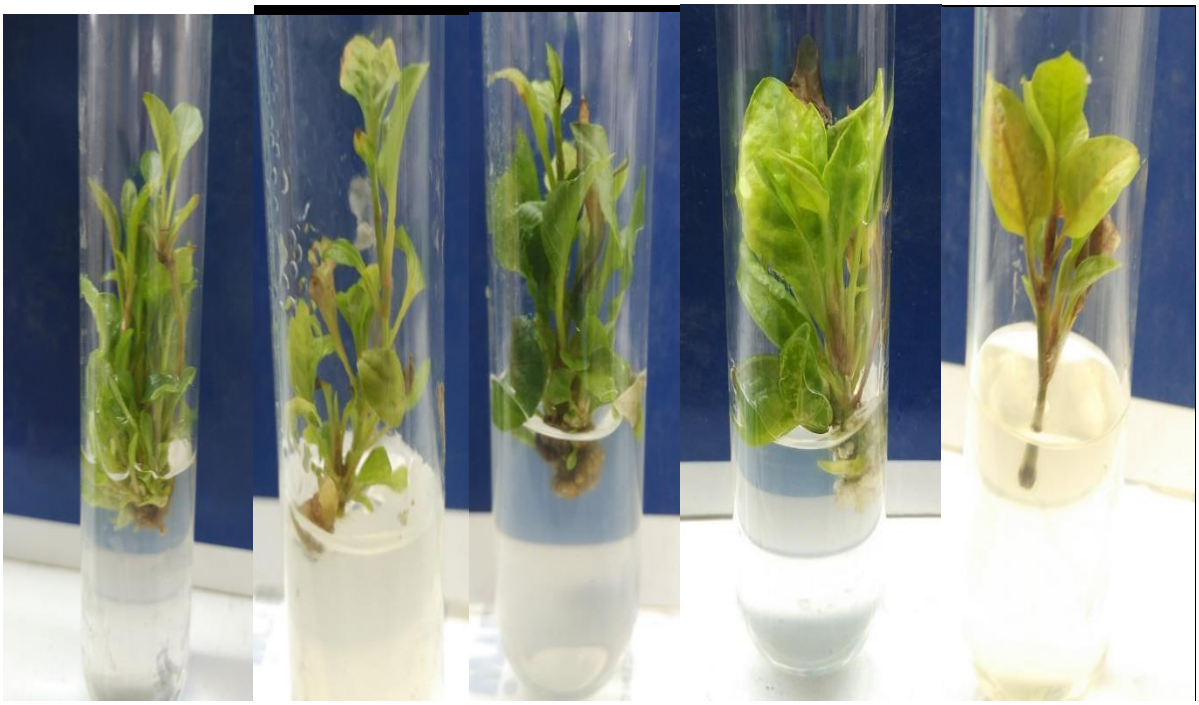
The data on influence of various media and carbon sources on plant height, number of nodes and number of shoots in five different accessions of *P. zeylanica* (524441, 398891, 439212, 539866, and 421418) have been furnished in Table 1 and 2. The plant height was significantly higher in Nitsch media in accession 398891 (3.969 cm). But the maximum number of shoots (06) and number of nodes (19) were observed in MS media in same accession with shoot length of 3.839cm. Among the different media MS media showed significantly better growth in terms of number of nodes and shoots in all the accessions of P. zeylanica followed by Nitsch, B5 and White media respectively. 398891 being the best among all accessions followed by 524441, 421418, 539866, 439212 respectively.

On the basis of various carbon source, it can be seen that highest shoot length (4.194 cm) is observed in sucrose in the accession 421418, also the number of nodes (17) were maximum in the same accession. While the highest number of shoots (4) were seen in both 524441 and 398891. On analyzing the data it can be seen that sucrose (10-17 nodes, 3-4 shoots) showed maximum effect on plant growth along with increased number of nodes and shoots comparable to that of glucose and fructose. Carbon sources are known to influence growth of callus (Kavi Kishor & Mehta 1987) as well as secondary metabolites like lignins in *Ipomoea* (Paska *et al*. 1999).

Table-1: Effect of different media on number and length of regenerated shoots, number of nodes in five different accession of *Plumbago zeylanica* after eight weeks of inoculation. Values are expressed as mean ± Standard Error (M ± SE). MS: Murashige and Skoog medium.

Experiment detail	Accession Number	Number of Nodes (M±SE)	Number of Shoots (M±SE)	Length of Shoots (cm) (M±SE)
WHITE	524441	13±1	3±1	3.244±0.1084

MEDIA	398891	11±2	3±0	2.822±0.2
	439212	9±2	3±1	2.486±0.22
	539866	7±2	3±0	3.588±0.733
	421418	12±2	3±2	2.664±1.377
NITSCH MEDIA	524441	16±2	3±0	3.688±0.554
	398891	13±2	3±1	3.969±0.5
	439212	4±1	2±0	1.9±0.18
	539866	10±2	3±1	2.572±0.256
	421418	9±2	3±1	2.836±0.143
MS MEDIA	524441	19±1	5±1	3.341±0.137
	398891	19±2	6±1	3.839±0.7
	439212	12±2	5±1	2.928±0.269
	539866	13±1	4±2	3.236±0.648
	421418	17±2	5±2	3.694±0.224
B5 MEDIA	524441	13±1	2±1	2.922±0.491
	398891	12±1	5±0	3.48±0.2
	439212	5±1	1±0	3.933±1.65
	539866	9±1	2±1	2.966±1.123
	421418	10±2	3±2	2.5±0.529



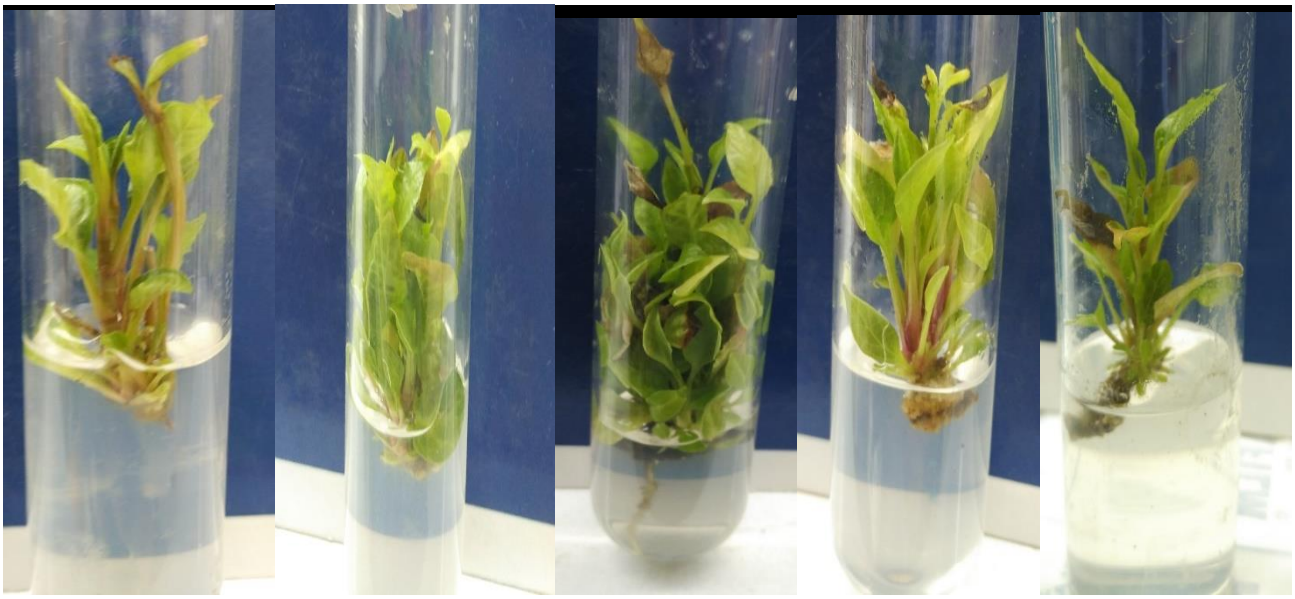
Acc. 398891 Acc. 524441 Acc. 421418 Acc. 439212 Acc. 539866

Figure-1: *In vitro* culture of five different accession of *Plumbago zeylanica* in MS media (After eight weeks of inoculation)

Table-2: Effect of different carbon sources on number and length of regenerated shoots, number of nodes in five different accession of *Plumbago zeylanica* after eight weeks of inoculation. Values are expressed as mean ± Standard Error (M ± SE). MS: Murashige and Skoog medium.

Experiment detail	Accession Number	Number of Nodes (M±SE)	Number of Shoots (M±SE)	Length of Shoots(cm) (M±SE)
SUCROSE	524441	15±3	4±2	3.478±0.411
	398891	15±2	4±2	3.372±0.769
	439212	10±2	3±1	3.180±0.114
	539866	14±2	3±1	3.047±0.298

	421418	17±2	3±1	4.194±0.558
GLUCOSE	524441	12±1	2±2	3.35±0.278
	398891	11±1	4±0	3.25±0.043
	439212	8±1	2±1	2.805±0.223
	539866	12±2	1±0	3.3±1.113
	421418	9±2	2±0	3.067±0.381
FRUCTOSE	524441	11±2	2±1	3.672±1.275
	398891	9±2	3±2	2.317±1.130
	439212	7±3	2±0	2.467±0.728
	539866	9±1	2±1	3.616±0.682
	421418	9±1	2±0	3.333±0.650



Acc. 398891 Acc. 524441 Acc. 421418 Acc. 439212 Acc. 539866

Figure-2: *In vitro* culture of five different accession of *Plumbago zeylanica* in MS media containing Sucrose as carbon source (After eight weeks of inoculation)

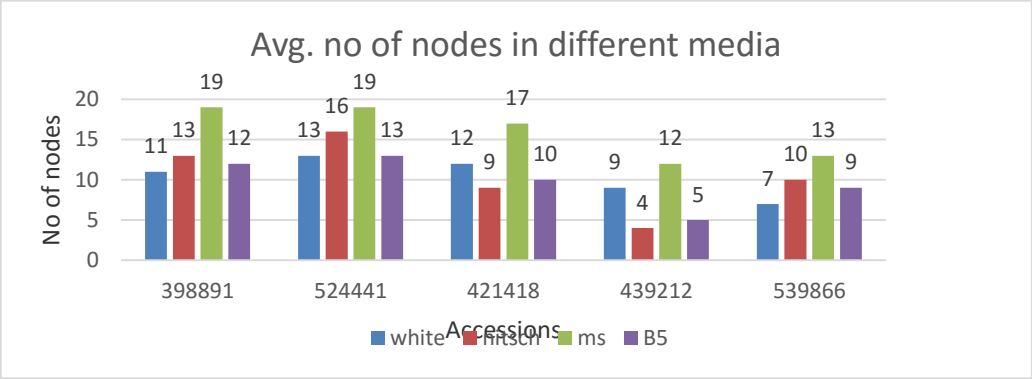


Figure-3: Effect of different media on Number of nodes in five different accessions of *Plumbago zeylanica*

Figure-4: Effect of different media on Number of shoots in five different accessions of *Plumbago zeylanica*

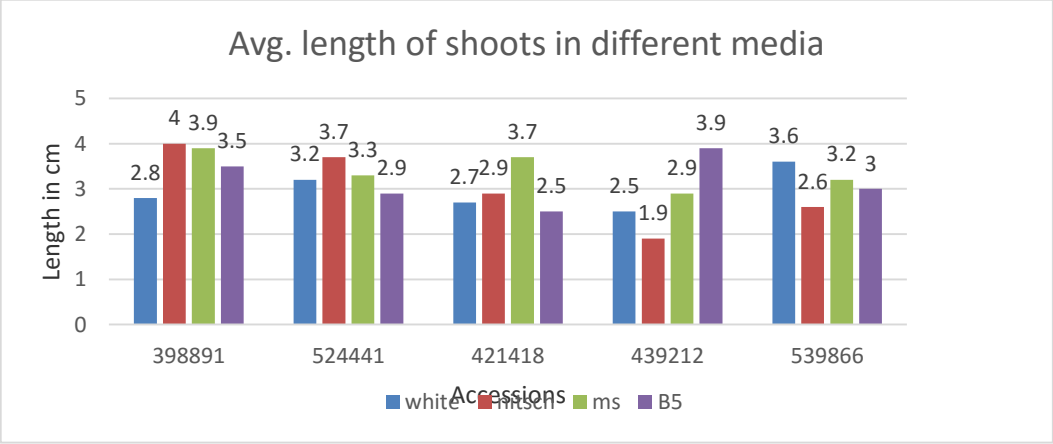


Figure-5: Effect of different media on length of shoots in five different accessions of *Plumbago zeylanica*

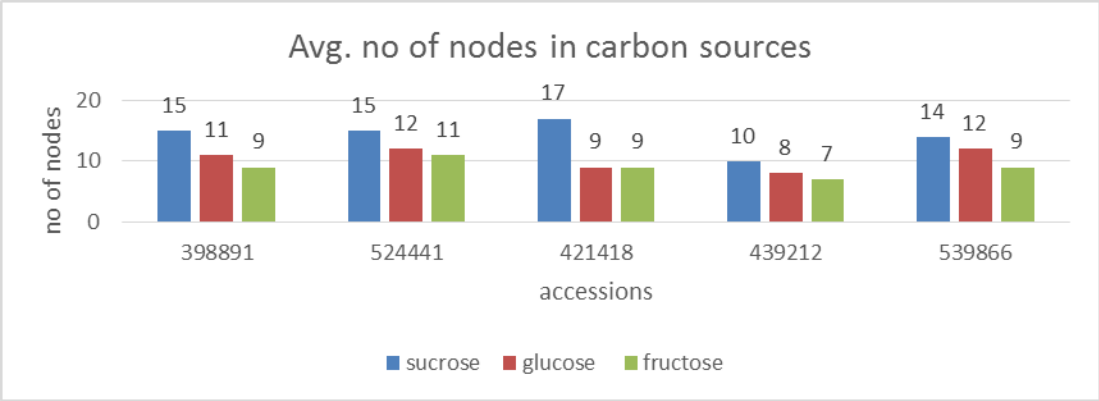


Figure-6: Effect of carbon sources on Number of nodes in five different accessions of *Plumbago zeylanica*

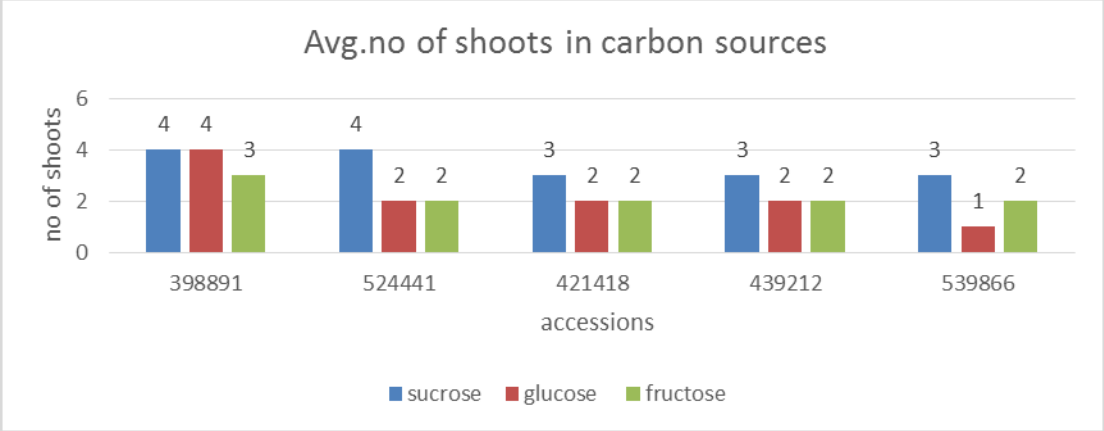


Figure-7: Effect of carbon sources on Number of shoots in five different accessions of *Plumbago zeylanica*

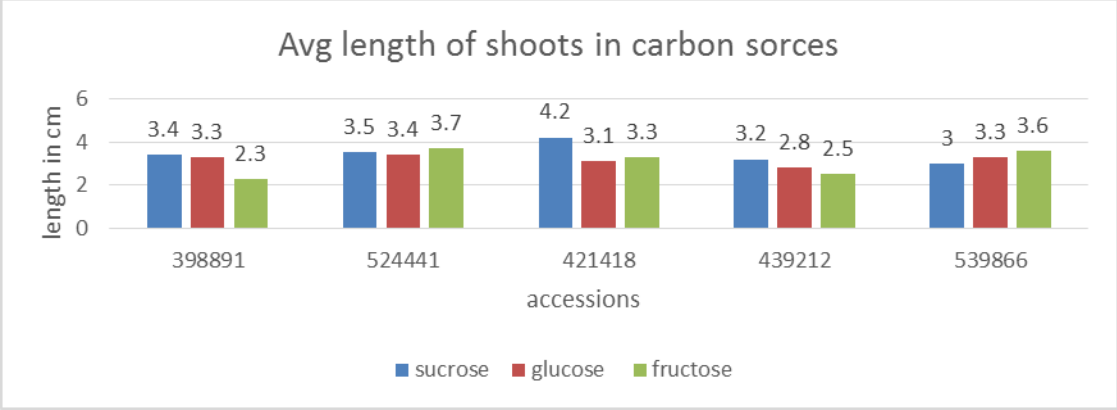


Figure-8: Effect of carbon sources on length of shoots in five different accessions of *Plumbago zeylanica*

Estimation of plumbagin by HPLC Method

After the treatment of five accessions with elicitors it was found that 524441 showed maximum growth in MS containing yeast extract (0.5mg/ml) phenotypically. It was taken for Plumbagin quantification as well as the relative percentage in cultures containing yeast extract in comparison to those without yeast extract i.e. only MS supplemented with BAP was evaluated by reverse phase high performance liquid chromatography (RP-HPLC). Roots were used as explants for the preparation of samples. Analysis of the methanolic extract of the root sample of *Plumbago zeylanica* accession no 524441 was done.

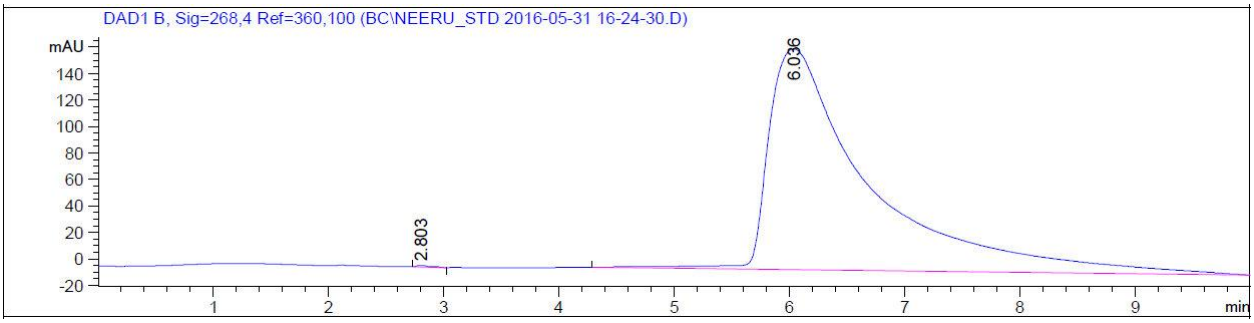


Figure-9: ***Chromatogram of standard Plumbagin from Plumbago zeylanica (L.)***

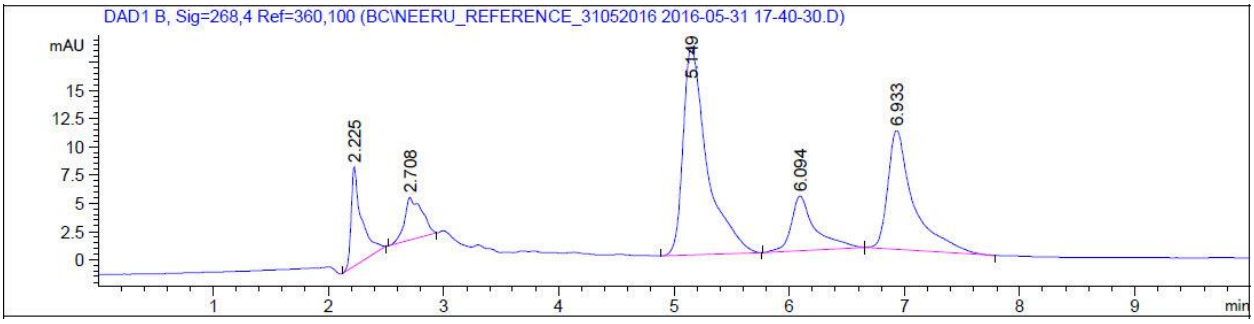


Figure-10: ***Chromatogram of Plumbago zeylanica root extract grown in MS media***

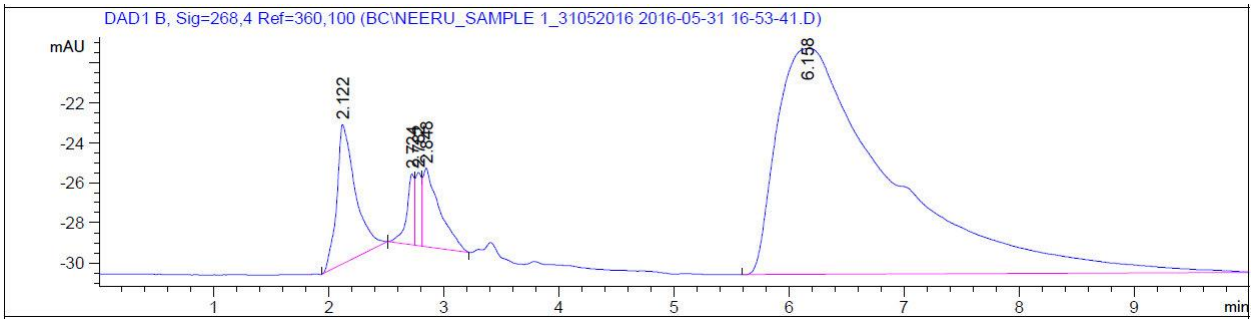


Figure-11: ***Chromatogram of Plumbago zeylanica root extract grown in MS media supplemented with yeast Extract.***

The amount of Plumbagin present in the extract was obtained by comparing the peak area from the standard. Also, the enhancement of Plumbagin can be determined by comparing the extract with the reference sample i.e., extract of plant without any elicitor (grown in MS media without any elicitor). Plumbagin was identified in extracts by comparing the retention time with that of the standard sample.

Figure 4 shows the chromatogram of standard plumbagin and its retention time is 6.036 minutes. If we compare the two chromatograms (figure 5 and 6) results, we can see that methanolic extract of yeast extract treated sample contain 84 percent of plumbagin whereas without treated sample contain 27 percent of plumbagin. The above data clearly reveal that due to the treatment of plant with the elicitor Yeast extract, it enhances the relative percentage of plumbagin approximately 3 times.

Fatty acid analysis by GC-MS

Table 6 lists the name of fatty acid as well as their relative percentage composition obtained from the gas chromatography mass spectrometry (GC-MS) analysis of the n- hexane extracts of five different accessions of *Plumbago zeylanica.* The plant sample contain various fatty acids, among which Octadecadienoic acid (8-22%), Octadecatrienoic acid (7-24%), Pentadecanoic acid (11-22%) being present in maximum amounts as per the chromatograms obtained.

Tetradecanoic acid (0-2%), Hexadecanoic acid (1-2%) and Octadecanoic acid (1-7%) were also obtained but comparatively low amount in all the five accessions of *P.zeylanica.*

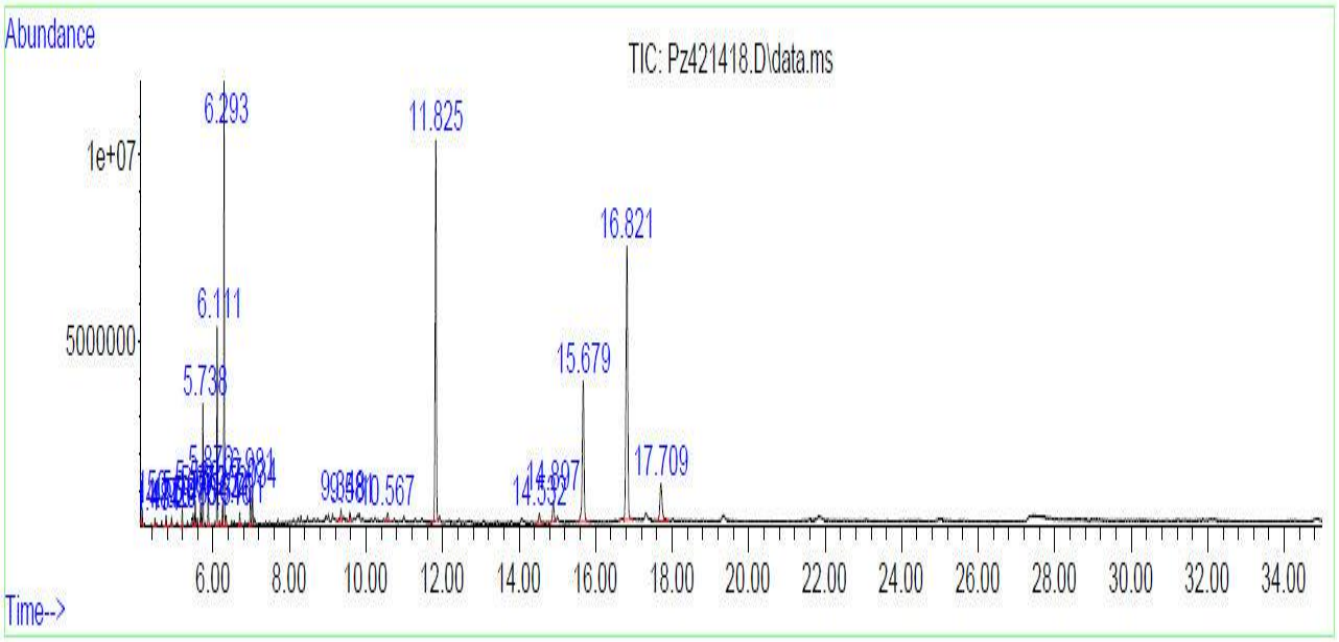


Figure 12: Chromatogram of *Plumbago zeylanica* accession no - 421418.

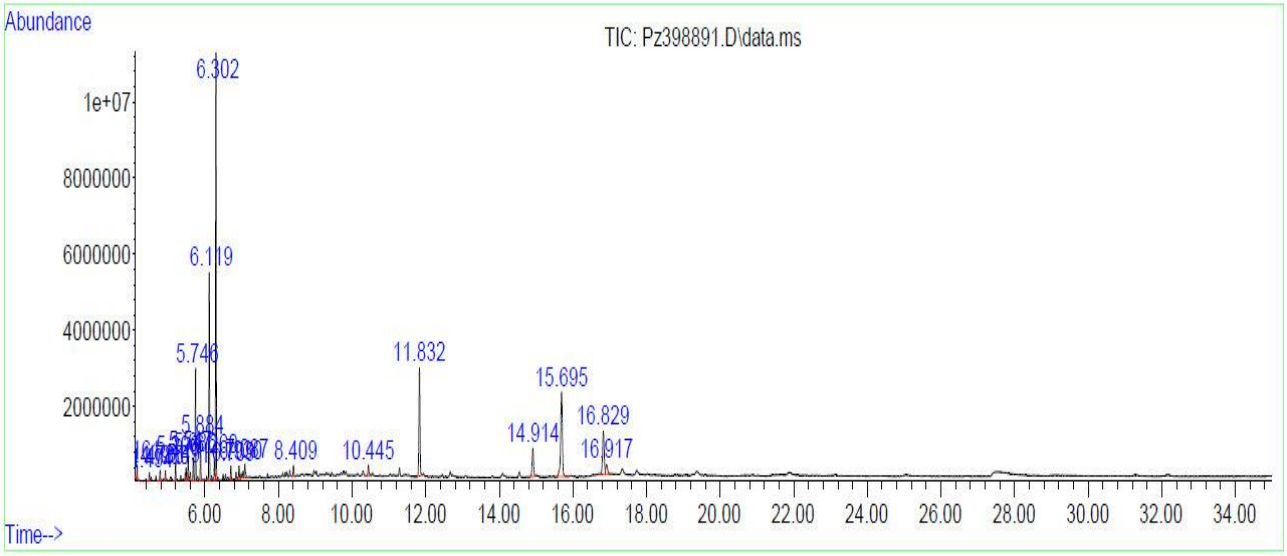


Figure 13: Chromatogram of *Plumbago zeylanica* accession no - 398891.

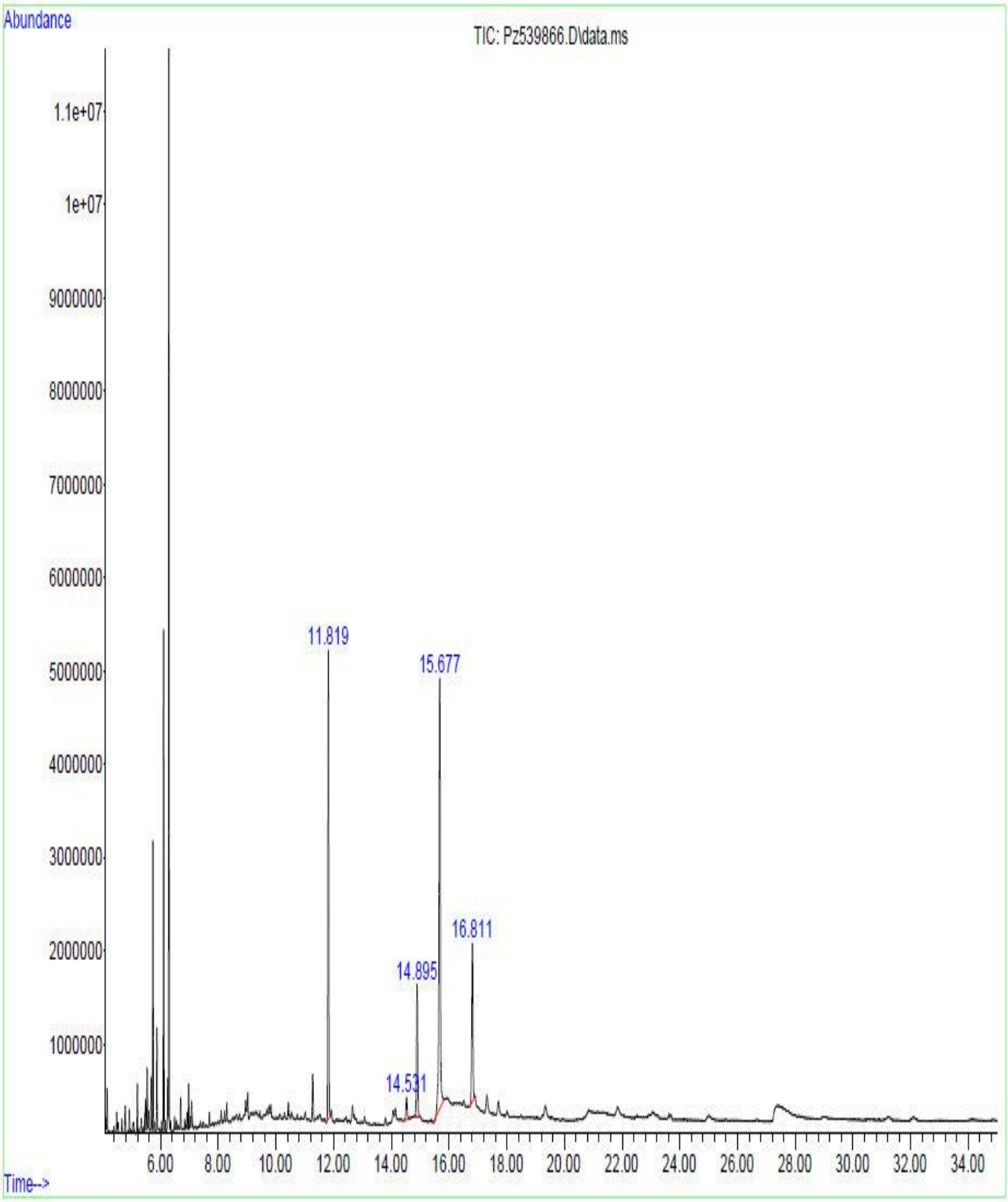


Figure 14: Chromatogram of *Plumbago zeylanica* accession no - 539866.

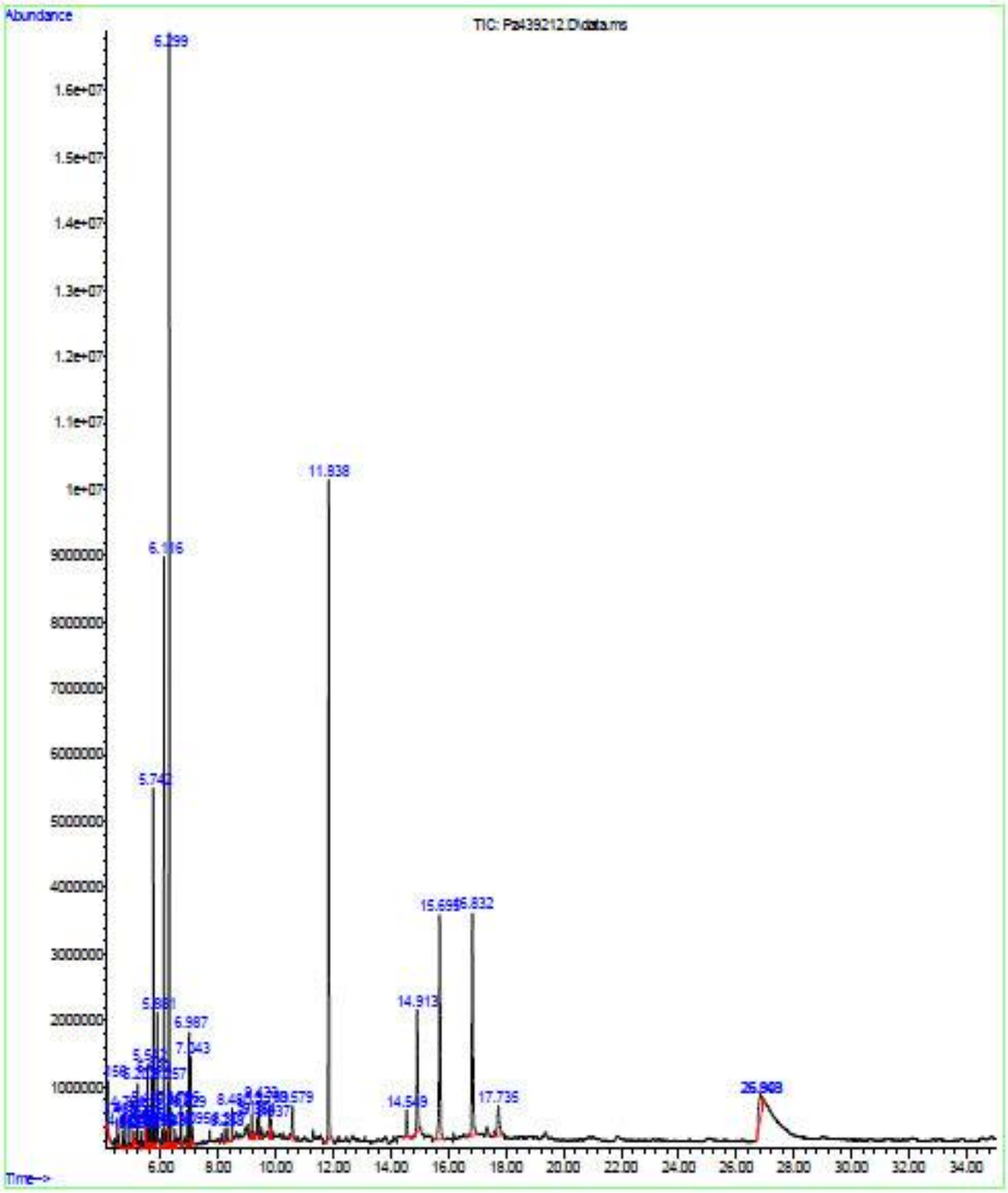


Figure 15: Chromatogram of *Plumbago zeylanica* accession no - 439212.

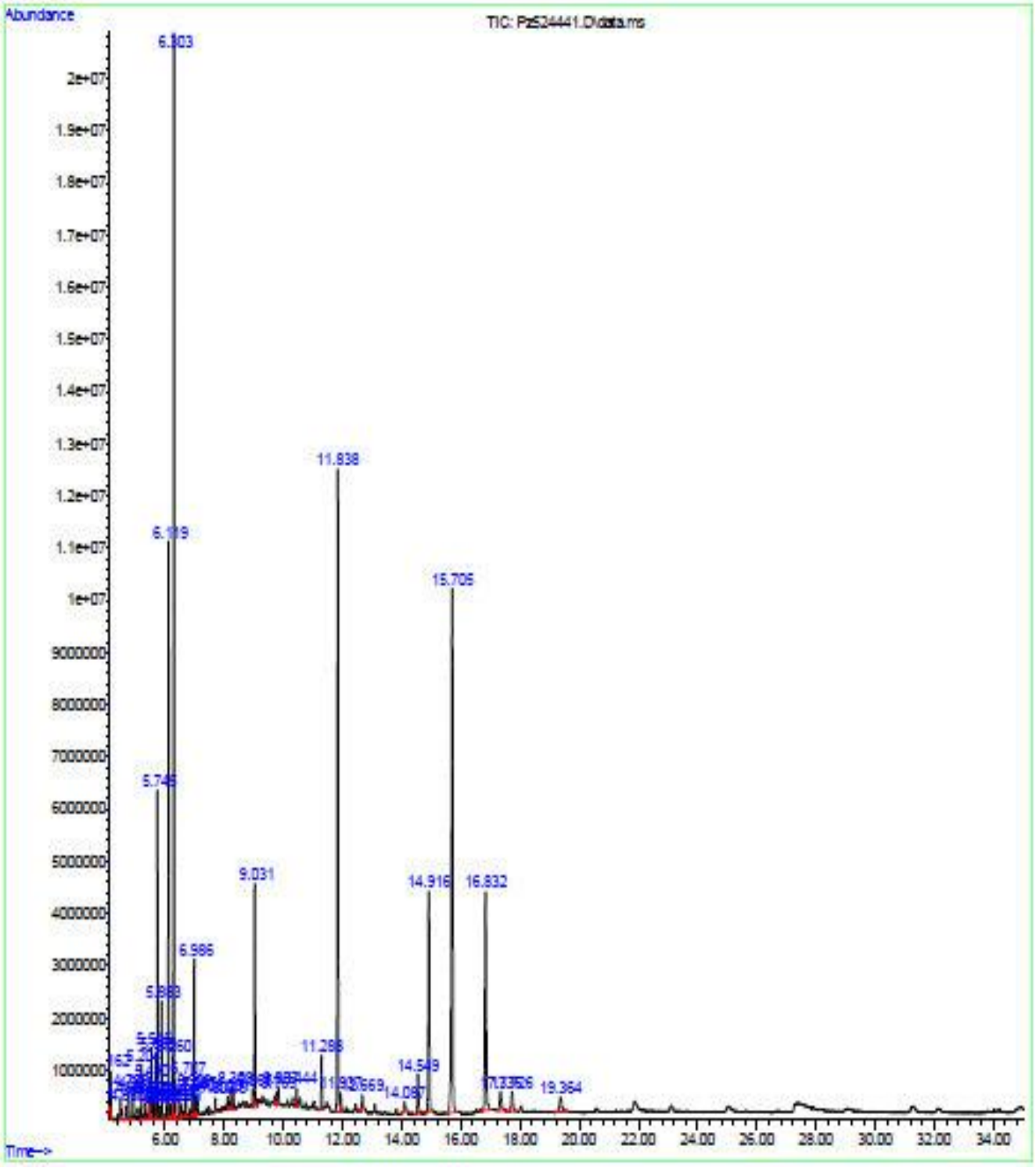


Figure 16: Chromatogram of *Plumbago zeylanica* accession no - 524441.

Table 4: Percentage of methylated fatty acids of five different accession of *Plumbago zeylanica*.

Fatty Acid	Relative %age Content of Fatty acid				
	Accession No. 421418	Accession No. 398891	Accession No. 539866	Accession No. 439212	Accession No. 524441
Tetradecanoic acid	0	1.16	0	0	0.47
Pentadecanoic acid	22.33	11.62	15.69	17.66	15.27
Hexadecanoic acid	0	0	1.45	2.18	1.12
Octadecanoic acid	1.57	4.17	5.87	4.16	6.65
9,12 Octadecadienoic acid	12.38	14.57	22.46	8.87	18.45
9,12,15 Octadecatrienoic acid	23.86	7.004	7.53	9.05	7.85
Total	**60.14**	**38.524**	**53**	**41.92**	**49.81**

The analysis of fatty acid from five different accession of *Plumbago zeylanica* by GC-MS showed that it contains various bioactive constituents including Pentadecanoic acid, Octadecadienoic acid, Octadecatrienoic acid, in major concentration. Hexadecanoic acid and Tetradecanoic acid are present in very less amount. From the chromatogram data of the table no. 4 shows that accession no 524441 contain maximum number of fatty acids compared to the other accessions. Unsaturated fatty acids, Octadecadienoic and Octadecatrienoic acid are the most important essential fatty acids as our body cannot synthesize these fatty acids. Accession no. 539866 contain highest percent of Octadecadienoic acid (approx.23%) followed by 524441 (18.45%), 398891 (14.57%), 421418 (12.38%) and 439212 (8.87%). When we consider the presence of Octadecatrienoic acids, it is recorded that accession no. 421418 contain the highest percent approx. 24 % followed by 43921 (9.05%), 524441 (7.85%), 539866 (7.53%) and 398891 (7.004%). Linoleic acid (Octadecadienoic acid) is essential for maintenance of growth and shown to be potent cycloxygenase-2 (COX-2) catalyzed prostaglandin biosynthesis inhibitors. Palmitic acid (Hexadecanoic acid) reduce the risk of cardiovascular disease. Stearic acid used in baked food items. Only three accessions of Plumbago zeylanica contain approximately 2 percent of Hexadecanoic acid (stearic acid). Thus it can be said that this plant has very good industrial value. It is also used in cholesterol-lowering diets.

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