Estimation of asiaticoside by RP-HPLC & FAME analysis by GC-MS of important medicinal plant *Centella asiatica*.

To be submitted as report of the major project II in the fulfillment of the requirement for the degree of

M. Tech.

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled **Estimation of asiaticoside by RP-HPLC & FAME analysis by GC-MS of important medicinal plant *Centella asiatica* (2k14/IBT/06)** in the 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 report of Major Project II entitled "**Estimation of asiaticoside by RP-HPLC & FAME analysis by GC-MS of important medicinal plant *Centella asiatica* (2k14/IBT/06)** in the 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 my own work carried out under the guidance of my project supervisor ***Dr. Navneeta Bharadvaja***, Assistant Professor, Plant Biotechnology, Department of Biotechnology, DTU. 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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CONTENTS

TOPIC: Estimation of asiaticoside by RP-HPLC & FAME analysis by GC-MS of important medicinal plant *Centella asiatica (L.)*

Tables, Graphs & Figures.

Estimation of asiaticoside by RP-HPLC & FAME analysis by GC-MS of important medicinal plant *Centella asiatica*.

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Abstract

The use of carbon and nitrogen in nutrient media is essential for growth and helps identify highly productive media for *Centella asiatica* (L.). It is used in traditional medicine for the treatment of various ailments. It contains various secondary metabolites which can be enhanced by the use of elicitors. Present study deals with the quantitative analysis of ethanolic extract of stem and leaf of this plant. An efficient and reproducible protocol was developed for micro propagation and enhances secondary metabolite production using explants of *Centella asiatica*. The combination of BAP, along with different concentration of sugars, nitrogen source and elicitors were used for this purpose. In between sucrose and fructose, sucrose containing media gives the better result than fructose. Among the nitrogen sources used ammonium nitrate shows best growth and malt extract shows best growth among the three different elicitors. Asiaticoside is one of the important phytocompounds that helps in the treatment of jaundice, missiles, hepatitis, small pox and rheumatism. Due to the importance of asiaticoside there is a need to micro propagate this plant so that maximum yield of asiaticoside can be achieved. Quantitative analysis of asiaticoside was performed using standard protocol by reverse phase high performance liquid chromatography. Fatty acids are of great significance when it comes to food nutrition evaluation, pharmacology and disease diagnosing. Saturated fatty acids helps to reduce cardiovascular risks and improving liver, lungs and brain health. Unsaturated (mono and polyunsaturated) fatty acids are used for declining heart disease and inflammation and increasing the immunity. Due to this medicinal importance, the analyses of fatty acids from five different accession of *Centella asiatica* were observed.

Introduction

Plants are gifted with various important and active phyto compounds such as vitamins, terpenoids, phenolic acids, lignins, tannins, flavonoids, alkaloids, amines, and other metabolites which are well to do in antioxidant activity (Zheng and Wang, 2001; Cai et al., 2003). Centella asiatica (L.) is a, creeping, perennial herbs, rooting at nodes and belongs to the family Apiaceaes. As because the plant inhabits in various region in India, it is popular with its regional names such as Thankuni in Bengali, Gotukola in Sinhali, Manimunni in Assam, Valleri in Decan, Mandookaparni in Hindi, Indian pennywort in English (Jamil et al., 2007), etc. The plant is found in abundance on moist, swampy, sandy or clay soils, often in large clumps, forming a dense green carpet.

The medicinal value of this plant was revealed in *Charaka Chikitsa* (Bhavna et al., 2011) and main active essential elements of C. asiatica (L.) are asiatic acid, madecasic acid, asiaticoside, madecassoside, brahmoside, brahmic acid, brahminoside, thankiniside, isothankunisode, centelloside, madasiatic acid, alkaloids, flavanoids, etc. (Glasby 1991, Bonfill et al., 2006, Zhang et al., 2008, Diallo et al., 1991, Inamdar et al.,1996, Jiang et al., 2005, Nhiem et al., 2011, Weng et al., 2012, Krishnamurthy et al., 2009, Subban et al., 2008, Veenrendra et al., 2002) which are known to take care of skin problems, to heal wounds, for stimulating the nerves and brain cells, antileprotic, antifilarial, antibacterial, adaptogenic, antifeedant, anti-stress, anti-ulcer, anti-oxidative stress, anti-radiation properties, anti-heavy metal poisoning, antiviral properties, etc. (Singh et al., 2010, Warrier et al., 1994, Soumyanath et al., 2005, Rao et al., 2006, Binns et al., 2002, Dhanasekaran et al., 2009, Sarma et al., 1996, Sarma et al., 1995, Gupta and Flora 2006, Flora and Gupta 2007, Hussin et al., 2007, Shinomol et al., 2010, Heong et al., 2011, Hamid et. al., 2002, Veenrendra et al., 2002, Sharma and Sharma 2002, 2005, Joy and Nair 2009, Boiteau et al., 1949, Saxena and Flora, 2006).

It is also reported that asiaticoside, one of the important compound of *C. asiatica* shows anti-tumor activity by apoptosis of tumor cells and is also used in the healing of leprosy or skin disorder by collagen I synthesis in human (Bonte et al., 1994). Due to it's medicinally important properties, this plant as well as its extract is in huge demand and such demand cannot be fulfilled by natural means. Thus in the search for alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have more prospective as enhancement to traditional production of bioactive plant metabolites (Ravishankar and Ramchandra Rao, 2002; Vanisree et al., 2004; Sharma et al., 2011).

Several groups have worked out that carbon and nitrogen source are 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. Treatment with elicitors is reported to enhance secondary metabolites in *C. asiatica* (Kim et al., 2004; Mangas et al., 2006; Prasad et al., 2013). Keeping these in mind following studies was done.

1) Tissue culture of *Centella asiatica*

Therefore, in the present investigation, focus has been done to *in vitro* micropropagation of the five different accession of *Centella asiatica* to find out the best culture conditions.

I. To find out the potential carbon source MS media containing different carbon sources (sucrose and fructose), supplemented with 6 Benzyl amino purine (BAP) were used separately.

II. To observe the most probable nitrogen sources MS media containing four different nitrogen sources (ammonium nitrate, potassium nitrate, sodium nitrate and calcium nitrate), supplemented with 6 BAP were tested individually.

III. To detect the effect of elicitors, MS media supplemented with three different elecitors [Malt extract (ME), jasmonic acid (JA) and salicylic acid (SA)] and BAP were used independently.

2) Estimation of asiaticoside

In the next phase of study was to estimate the asiaticoside on the best grown accession supplemented with elicitors using reverse phase high performance liquid chromatography (RPHPLC).

3) FAME analysis

Another attempt has been made to investigate the fatty acid methyl ester (FAME) profile of five different accession of *Centella asiatica* under normal environmental conditions.

The purpose of this investigation is to find out potential culture conditions as well as potential accession which can provide the high yield of plant material as well as active compounds. Quantitative analysis of asiaticoside was carried out using standard protocol of reverse phase high performance liquid chromatography with the plant's accession which provided best results with malt extract. FAME analysis was done with GC-MS for five different accessions to access the potential fatty acid containing accession of *Centella asiatica*.

Review of literature

Morphology

Centella asiatica (L.) is a slightly fragrant, perennial, creeper herb, rooting at nodes. It can attains height up to 15cm. Centella asiatica do well extensively in shaded, swampy, damp and wet places such as paddy fields, river banks forming a dense growth. The leaves are human brain shaped 1-3 from each node of stems. Flowers are in cluster, each umbel consisting of 3-4 white to purple or pink flowers. Flowering period of the plant is April-June. Fruits are approx 2 inches long, globular in shape and strongly thickened pericarp. (Singh et al., 2010)

Geographical area

Centella asiatica found throughout tropical and sub tropical regions of India, Srilanka, parts of China, Northern Australia, Western South Sea Islands, Madagascar, South Africa, South East USA, Mexico, Venezuela, Columbia and Eastern South America up to an altitude of 600m. (Gupta et al., 2007)

Chemical composition:

Centella asiatica contain various types of compounds:

Triterpene acids: Asiatic, madacassic, terminolic, centic, centellic, centoic acid, indocentoic acid, isobramhic, betulic, brahmic and madasiatic acids are there in Centella as triterpene acid. (Gupta et al., 2006)

Volatile and fatty oil: The plants contain various types of volatile and fatty oils. Glycosides of palmitic, stearic, oleic and linolenic acids are present in fatty oils. The plant also contains alkaloids, glycosides, flavonoids and various other compound.(Jamil et al., 2007)

Properties

Role as Antioxidant:

The plants are of great attention in food industry as it contains essential oils and various extract, which have the potential as natural additives in packaged food product. It can replace the chemical additives which have been used as antioxidant. The component of *Centella asiatica* has high potential in antioxidant activity (Hamid et al., 2002). Hashim et al. stated that antioxidant activity in Centella is roughly same as in vitamin C and in grape seed extract. Antioxidant activity of the plants were investigated and it was found that the plant extract were carry out scavenging the DPPH free radical and dropping ferric ions (Weng et al., 2012).

It was also found that the leaves of Centella shows high antioxidant activity using three different pathways, free radical scavenging activity, inhibition of peroxidation of linoleic acid and radical scavenging DPPH (Gupta et al., 2006)

According to Zainol, et al, among the different parts of *C. asiatica*, leaves showed highest antioxidant activity which also contains highest phenolic contents, when compare to other plant parts. This result suggests that phenolic compounds are the major contributors to the antioxidative activities of *C. asiatica*. On the other hand, Abdul- Hamid, et al., 2002 reported that ethanol extract of root of *C. asiatica* exhibited the highest activity though it was not significantly different from the leaves. The antioxidative activity of different parts of may be due to the reduction of hydroperoxides, inactivation of free radicals, chelation of metal ions or combinations thereof.

Oral treatment of crude methanol extract of *C. asiatica* on mice having lymphoma is applied and it was observed that there is significant increase in the superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx) which are responsible for antioxidant activity.

The role of asiaticoside in wound healing as antioxident was studied. It was reported that asiaticoside driven from *Centella asiatica* enhance the antioxidant level from the initial stage of healing It was also observed that the plant also contain antioxidant activity of carotenoid and ascorbate peroxidase. The level of antioxidant activity enhance as the concentration of Cenlella extract increased 1000 to 5000ppm. Two new flavonoids (castilliferol 1 and 2) were isolated from the whole plant of *Centella asiatica*. They exhibit antioxidant activity with DPPH radical solution (Subban et al., 2008).

Antibacterial activity:

Medicinal plants are popularly used in natural medicines because of their very low side effects and economic. *Centella asiatica* is very important as it perform antibacterial activity in opposition to a broad range of bacteria (Jagtap et al. 2005)

Micropropagation and enhance asiaticoside production in *Centella asiatica.*

Growth and triterpenoid production based on macronutrients were studied. Centella asiatica plant cells grown macronutrients, 5.05mM ammonium, 15.0 mM nitrate and 2.6 mM phosphate gives optimum cell dry weight (16g/L). But the production of triterpenoids was lower than the 4mg/g of cell dry weight (Omar et al., 2005).

Effect of growth regulators on *Centella asiatica* plant culture was studied. B5 media supplemented with 0.01mg/L 2,4-D reduce the growth and asiaticoside production in Centella. Growth medium supplemented with TDZ (cytokine) showed highest growth and asiaticoside production than other (BA, Zeatin and Kinetin) cytokines (Kim et al., 2004).

Effect of elicitors on asiaticoside production in *Centella asiatica* was studied. For this perpose Yeast extract, Methyl jasmonate (0.01mM), Cdcl2, CuCl2 were used. Among them MJ (0.1mM) shows highest asiaticoside production (116.8 mg/L). Cdcl2 and CuCl2 showed negative result on asiaticoside production (Kim et al., 2004).

Methyl jasmonate (100µM) supplemented media can enhance the growth and secondary metabolite production in variety of plant species. Results showed that the plants, *C. asiatica* and *G. glauca* enhance the triterpenoid content exogenous methyl jasmonate treated plant species (Mangas et al., 2006).

Several important high value compounds such as asiaticoside and madacassoside are shown produced from hairy root culture of *Centella asiatica* (L.) Urban. Co cultivation of the plant (transformed with Agrobacterium) with A. rhizogenes were done for seven days to obtain abundant hairy roots. The resulting transformed plants were treated with Methyl jasmonate for three weeks and the results showed high amount (7.12mg/g dry wt.) of asiaticoside production (Kim et al., 2007).

MS media supplemented with 2.5 mg/L kinetin showed shoot growth index 6.06 and highest asiaticoside production (3.8 mg/g dry wt.) in *Centella asiatica* (L.) on the 35th day. Shoot growth and asiaticoside production was influenced by the concentration of ammonium, nitrate and cupper present in the medium were observed. Total nitrogen upto 60 mM showed highest (8.7mg/g) level of asiaticoside accumulation. It was also observed that sucrose concentration (5%) significantly (7.2 mg/g of dry wt.) raise the asiaticoside production (Prasad et al., 2011).

A recent study investigated the elimental contents and fatty acids in four different medicinal plants (*Kaiempferia rotunda; Cuscuta reflexa; Centella asiatica; Asparagus racemosus*). Results showed that the plants contains considerable amounts of Na, K, Mg, Nn, Fe, Cu, Cr, Cd elements find out by Atomic Absorption Spectroscopy (AAS) and essential fatty acids such as heneicosanoic acid, pentadecanoic acid, hexadecanoic acid, heptadecanoic acid and octadecanoic acid verified by GC-MS.

Materials and Methodology

1) In vitro micro propagation of *Centella asiatica*

Collection of plant material

Cultures of five different accessions i.e. 281374, 383913, 342109, 347492, 331514 of *C. asiatica* were collected from NBPGR New Delhi, India.

1) To study the effect of carbon source, nitrogen source and elicitors on shoot multiplication Murashige and Skoog (MS) media was used (Murashige et al., 1962). pH was adjusted to 5.8 using 1 N HCl or 1 N NaOH solution. 0.8 % plant agar was used to solidify the media. Sterilization of the media was done by autoclaving for 20 min at 121°C and 15 lb pressure.

I. For the experiment on carbon sources MS media was supplemented with 1.5 mg/L 6-Benzyl amino purine along with different carbon sources [sucrose (3%) and fructose (3%)] were prepared separately.

II. To find out the most effective nitrogen source on shoot multiplication and elicitors on shoot multiplication MS media supplemented with 1.5 mg/L 6- Benzyl amino purine along with different nitrogen sources [NH_4NO_3 (1650mg/L), KNO_3 (800mg/L), $NaNO_3$ (1650mg/L), $Ca(NO_3)_2$(825mg/L)] were get ready individually.

III. To detect the effect of elicitors, MS media supplemented with three different elecitors [Malt extract (1mg/L), Salicylic acid (1mg/L), Jasmonic acid (1mg/L)]and 1.5 mg/L BAP were used independently.

After the solidification of media, sterile explants from each accession were inoculated in culture tubes (25x150 mm) separately. The cultures were incubated at 26 ± 2°C under 16 h photoperiod and light intensity of 3000 lux for four weeks. Each and every experiment was done in triplicates. Visual data was recorded after 4th week of inoculation in terms of number of shoots and length of shoots for in vitro growth measurement.

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

2) Estimation of asiaticoside

For the analysis of asiaticoside reverse phase high performance liquid chromatography (RP-HPLC) with UV detection at 220 nm were used.

Equipment of HPLC: HPLC system including: a pump, an injection port, column compartment, and UV- VIS detector. Fused core C18 HPLC column, Sonicator, Polytetrafluoroethylene (PTFE) syringe filter, 0.22 micrometer.

Standard preparation

Weigh 1 mg of asiaticoside standard dissolve in 10 ml of methanol.

Preparation of sample for estimation of asiaticoside using HPLC

- Weight 75mg of air dried powdered leaf sample into the glass tube.
- Add 20 ml of methanol and sonicate the flask for 10 min.
- Occasional shaking is done.
- After cooling filter the sample with 0.45 micrometer filter paper.
- Final volume makes up to 30 ml with methanol and mix well.
- Filter a portion of solution through a 0.2 micrometer polytetrafluoroethylene (PTFE) syringe filter and inject into HPLC.

Chromatographic conditions

Column	C18.
Mobile Phase	Water : Methanol (30:70)
Flow rate	1ml/min
Inj Volume	15 microliter
Column Temp.	26^0C
Detection	UV at 220nm.

Procedure for HPLC

Analysis was carried out by HPLC system. First of all standard solution of asiaticoside was injected, after that basal sample solution without any elicitor treatment and lastly the treated samples with malt extract elicitor were injected which were prepared in triplets. Each sample required 10 minutes to run.

3) FAME analysis

Preparation of sample for fatty acid analysis using GC-MS

- Leaves of *Centella asiatica* were transferred to a screw-cap (teflon coated) glass tube.
- Added 1ml of 2% methanolic HCl to chopped leaves.
- The sample was then incubated at 90^{o}C for an hour.
- After that 1 ml of 0.9% Nacl was added followed by 2 ml of hexane and centrifuged at 2000 rpm for 2 minutes.
- The upper (hexane) layer of the sample was transferred into a fresh glass tube and dried under nitrogen flow.
- Dried sample was then diluted with 100 micro liter of hexane.
- 1 micro liter of samples was then injected into the GC-MS for analysis

Methodology used in GC-MS analysis

Analysis was carried out by GC-MS electron impact ionization method. A gas chromatograph coupled mass spectrometer. The carrier gas consisted of helium. The column temperature was maintained 50 to 250^{0}C. It requires 35 min to run each sample.

Results and Discussion:

1) In vitro micro propagation of *Centella asiatica*

Effect of carbon source, nitrogen sources and elicitors on shoot regeneration was observed in five accessions of *Centella asiatica*. Observation was recorded as mean value of triplicate samples after four weeks of inoculation (Table-1, 2 & 3).

I. Effect of Carbon sources

Two different carbohydrate sources i.e. sucrose and fructose were tested for their potential on shoot regeneration in five different accession of *Centella asiatica* and it was found that sucrose showed better shoot growth than fructose in different accession of *Centella asiatica*.

S.No	Accession Number	Treatment	No. of shoots (M±SE)	Length of shoot(cm.) (M±SE)
1	Accession no.281374	MS+Sucrose+ BAP(1.5mg/ltr)	8±1.15	1±0.06
		MS+Fructose+BAP(1.5mg/ltr)	2±0.57	1.5±0.16
2	Accession No.383913	MS+Sucrose+ BAP(1.5mg/ltr)	3±0.57	0.27±0.04
		MS+Fructose+BAP(1.5mg/ltr)	2±0.57	0.75±0.08
3	Accession No.342109	MS+Sucrose+ BAP(1.5mg/ltr)	5±1.2	0.52±0.02
		MS+Fructose+BAP(1.5mg/ltr)	2±0.57	0.75±0.10
4	Accession No. 347492	MS+Sucrose+ BAP(1.5mg/ltr)	16±3.05	1.66±0.15
		MS+Fructose+BAP(1.5mg/ltr)	5±0.57	0.7±0.05
5	Accession No. 331514	MS+Sucrose+ BAP(1.5mg/ltr)	10±1.15	1.5±0.08
		MS+Fructose+BAP(1.5mg/ltr)	3±0.57	1±0.09

Table-1: Effect of different carbon sources on number and length of regenerated shoots in five different accession of *Centella asiatica* after four weeks of inoculation. Values are expressed as mean ± Standard Error (M ± SE). MS: Murashige and Skoog medium; BAP: 6-Benzyl amino purine.

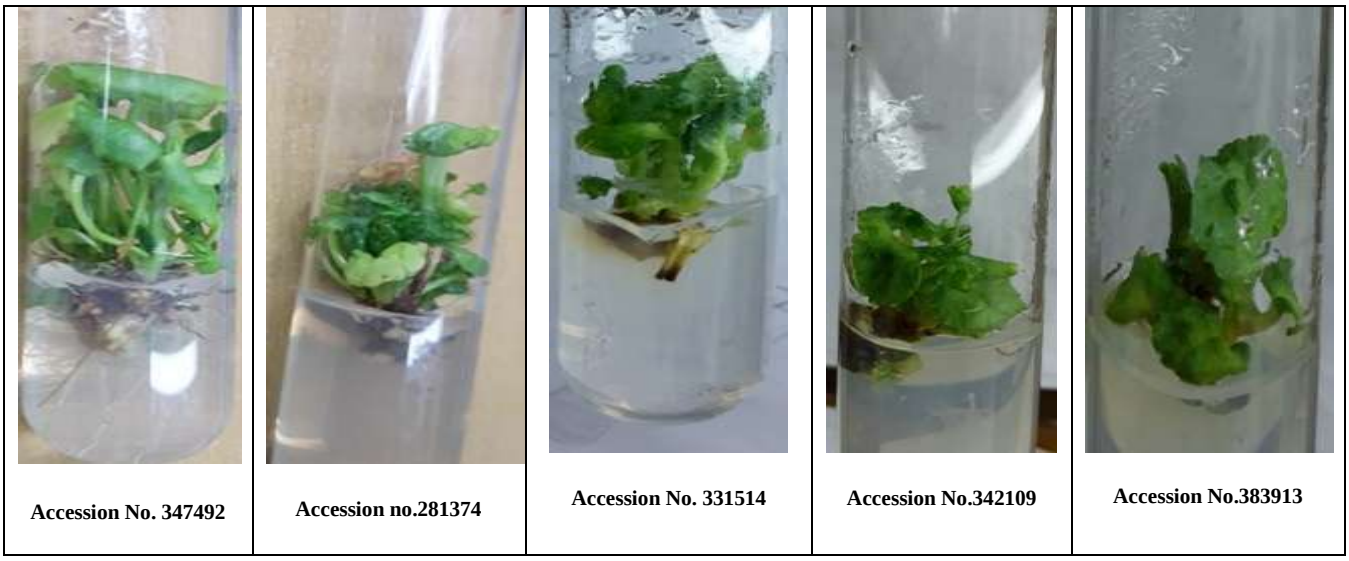


Figure-1: *In vitro* culture of five different accession of *Centella asiatica* in MS media containing Sucrose (After four weeks of inoculation)

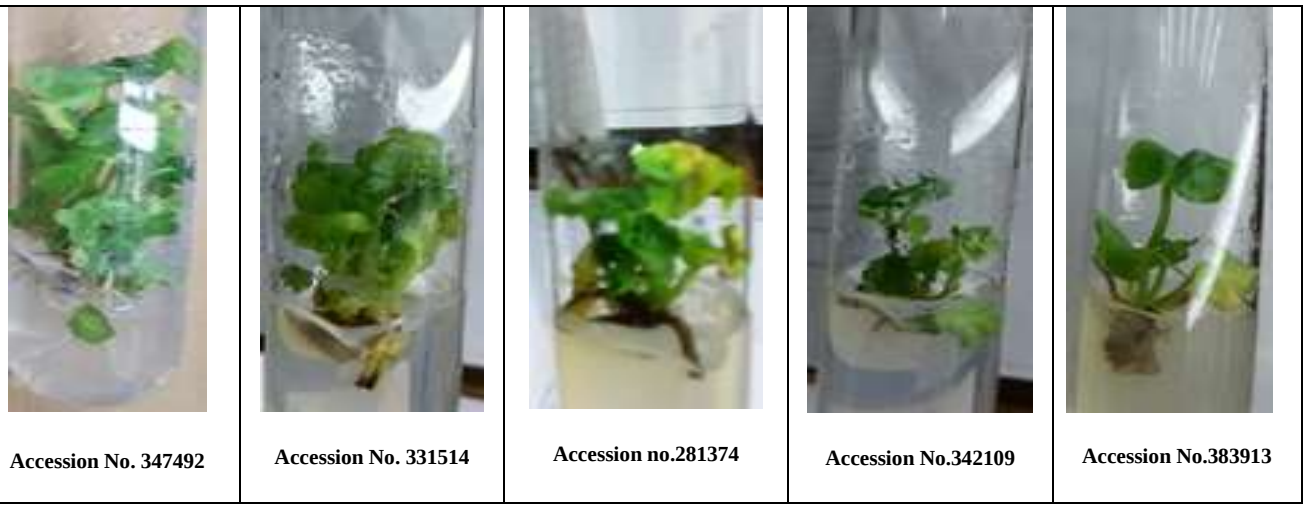
Maximum number of shoots generation was recorded in MS media supplemented with Sucrose (3%). In case of sucrose as carbon source, maximum number of shoots was reported in accession number 347492(16) followed by 331514 (10), 281374 (8), 342109 (5) and 383913 (3). Maximum average length was reported in accession number 347492 (1.66).

II Effect of nitrogen sources

A comparative study with four different nitrogen sources i.e. ammonium nitrate, potassium nitrate, sodium nitrate and calcium nitrate were tested for their potential on shoot regeneration in five different accession of *Centella asiatica*. Each accessions response in its own way in MS media containing different nitrogen sources. Accession no 383919 shows maximum average no of shoots in the media containing NaNO3 (9.33) followed by KNO3 (7.66), NH4NO3 (4.67), Ca (NO3)2 (0.25) and maximum average length of shoots generation was reported in KNO3 containing media (1.46) followed by NH_4NO_3 (1.4), $NaNO_3$ (1.17), Ca $(NO_3)_2$ (0.25). Accession no 281374 also shows highest no of shoot in $NaNO_3$ (8) followed by NH_4NO_3 (6.33), Ca $(NO_3)_2$ (4.66), KNO_3 (3) but average length of shoot was maximum in KNO_3 (1.4) containing medium. In the accession no 331514 and 347492 maximum average no of shoots generation was reported in NH_4NO_3 containing media 10 and 14.66 respectively. Highest average length of shoots was recorded in the media containing $NaNO_3$ (1.94) followed by NH_4NO_3 (1.16), Ca $(NO_3)_2$ (1.04), KNO_3 (0.93) in the accession no 347492. Whereas accession no 342109 shows a different type of result, here KNO_3 containing media shows highest no of shoots (7.66) followed by NH_4NO_3 (5.33) $NaNO_3$ (4.66), Ca $(NO_3)_2$ (3.33). Among five different accessions 347492 shows the best result in presence of MS media supplemented with separate nitrogen sources followed by accession no

S.No.	Accession no.	Nitrogen Source Treatment	No of shoots (M ±SE)	Length of shoots in c.m (M±SE)
1	383913	Ammonium Nitrate	4.67± 0.66	1.4±0.33
		Potassium Nitrate	7.66±2.18	1.46±0.41
		Sodium Nitrate	9.33±2.4	1.17±0.38
		Calcium Nitrate	3±0.57	0.25±0.06
2	281374	Ammonium Nitrate	6.33±0.88	1.23±0.26
		Potassium Nitrate	3±0.57	1.35±0.23
		Sodium Nitrate	8±0.1.5	0.76±0.17
		Calcium Nitrate	4.66±0.66	1.01±0.23
3	331514	Ammonium Nitrate	10±1.15	1.49±0.27
		Potassium Nitrate	7±0.57	0.9±0.22
		Sodium Nitrate	4±0.57	0.75±0.14
		Calcium Nitrate	5.6±1.2	0.97±0.36
4	347492	Ammonium Nitrate	14.66±2.4	1.61±0.59
		Potassium Nitrate	5.66±0.88	0.93±0.29
		Sodium Nitrate	10±1.15	1.94±0.53
		Calcium Nitrate	5.66±1.2	1.04±0.33
5	342109	Ammonium Nitrate	5.33±0.88	1.37±0.62
		Potassium Nitrate	7.66±1.2	0.81±0.3
		Sodium Nitrate	4.66±0.66	0.64±0.19
		Calcium Nitrate	3.33±0.88	0.84±0.21

Table-2: Effect of different nitrogen sources on number and length of regenerated shoots in five different accession of *Centella asiatica* after four weeks of inoculation. Values are expressed as mean ± Standard Error (M ± SE). MS: Murashige and Skoog medium; BAP: 6-Benzyl amino purine.



Accession No. 347492 | **Accession No. 331514** | **Accession no.281374** | **Accession No.342109** | **Accession No.383913**

Figure-2: *In vitro* culture of five different accession of *Centella asiatica* containing Ammonium Nitrate as Nitrogen source. (After four weeks of inoculation)

331514, 383913, 281374 and 342109 respectively. It was found that ammonium nitrate containing media showed maximum average length of shoot regeneration than other three nitrogen sources in different accession of *Centella asiatica*.

III Effect of elicitors

Among the elicitors used, MS media supplemented with malt extract (1mg/ltr) showed maximum number and maximum length of shoots. Maximum effect of malt extract was found on accession no. 281374 as maximum number of shoots i.e. 25 were reported in this accession followed by 342109 (16), 331514 (15), 347492 (14) and 383913 (10). Maximum average length was reported in accession number 347492 (3.07) followed by 281374 (2.35), 342109 (1.69), 331514 (1.75), 383913 (1.5).

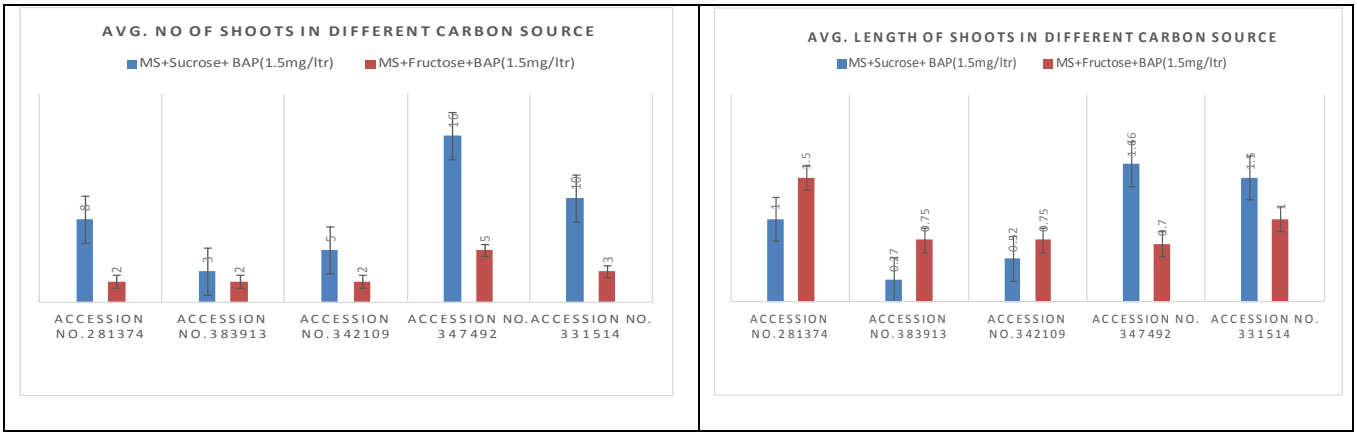
S.No	Accession Number	Treatment	No. of shoots (M±SE)	Length of shoot(cm.) (M±SE)
1	Accession no.281374	MS+ BAP(1.5mg/ltr)+ME(1mg/ltr)	25±1.52	2.35±0.12
		MS+ BAP(1.5mg/ltr)+SA(1mg/ltr)	4±1.15	1±0.08
		MS+ BAP(1.5mg/ltr)+JA(1mg/ltr)	2±1	0.75±0.12
2	Accession No.383913	MS+ BAP(1.5mg/ltr)+ME(1mg/ltr)	10±0.57	1.3±0.05
		MS+ BAP(1.5mg/ltr)+SA(1mg/ltr)	1±0.33	1.5±0.12
		MS+ BAP(1.5mg/ltr)+JA(1mg/ltr)	1±0	0.5±0.05
3	Accession No.342109	MS+ BAP(1.5mg/ltr)+ME(1mg/ltr)	16±3.46	1.69±0.09
		MS+ BAP(1.5mg/ltr)+SA(1mg/ltr)	1±0	2.5±0.28
		MS+ BAP(1.5mg/ltr)+JA(1mg/ltr)	2±0.57	0.75±0.07
4	Accession No. 347492	MS+ BAP(1.5mg/ltr)+ME(1mg/ltr)	14±1.15	3.07±0.12
		MS+ BAP(1.5mg/ltr)+SA(1mg/ltr)	5±1.15	0.9±0.10
		MS+ BAP(1.5mg/ltr)+JA(1mg/ltr)	5±1.52	0.52±0.03
5	Accession No. 331514	MS+ BAP(1.5mg/ltr)+ME(1mg/ltr)	15±1.15	1.43±0.07
		MS+ BAP(1.5mg/ltr)+SA(1mg/ltr)	8±2	1.75±0.07
		MS+ BAP(1.5mg/ltr)+JA(1mg/ltr)	12±2.88	1.66±0.07

Table-3: Effect of different elicitors on number and length of regenerated shoots in five different accession of *Centella asiatica* after four weeks of inoculation. Values are expressed as mean ± Standard Error (M ± SE). MS: Murashige and Skoog medium; BAP: 6-Benzyl amino purine.

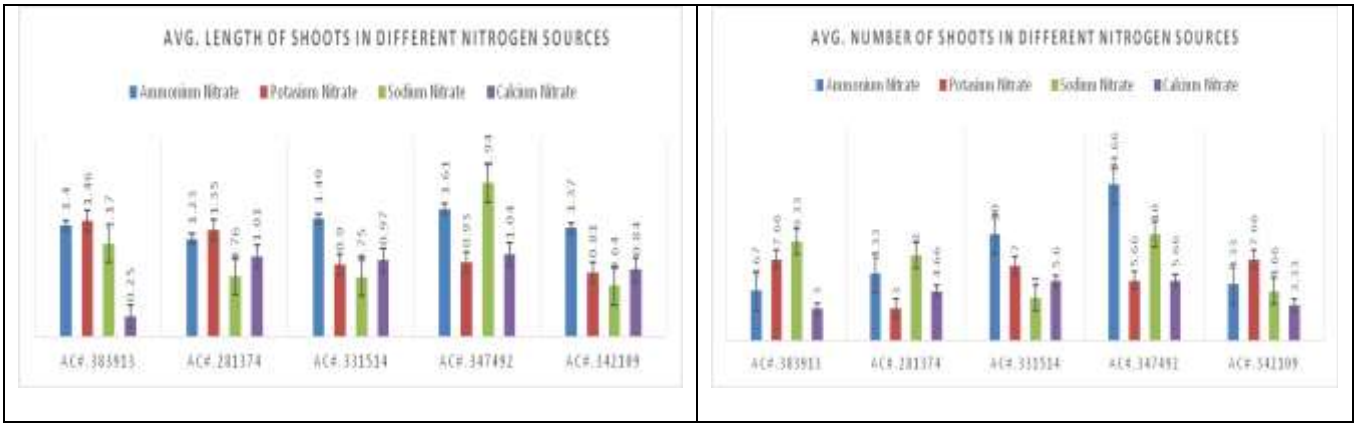
Accession no.281374	Accession No.383913	Accession No.342109	Accession No. 347492	Accession No. 331514

Figure-3: *In vitro* culture of five different accession of *Centella asiatica* in malt extract (After four weeks of inoculation).

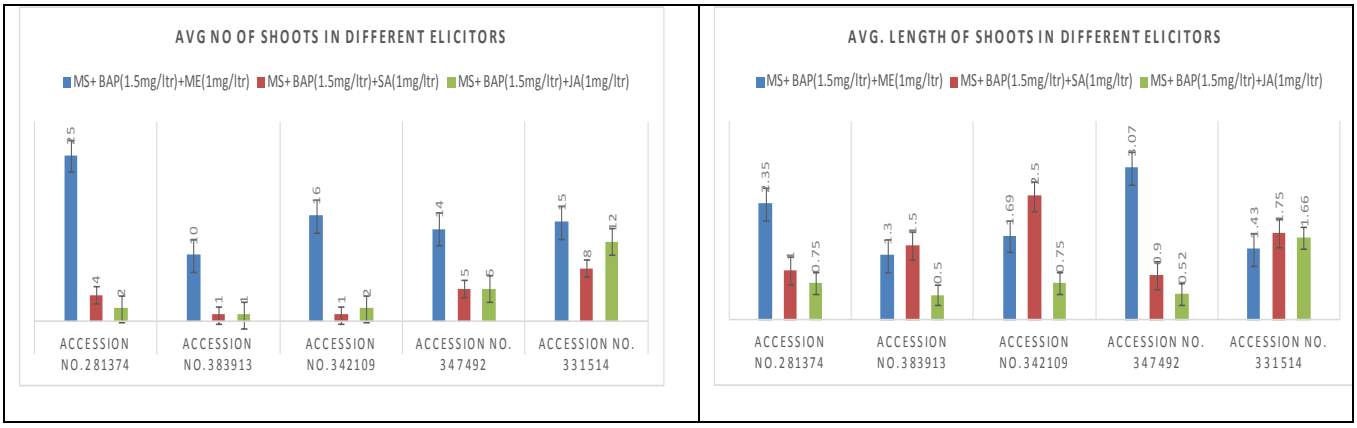
Graphical Representation of data.



Graph-1: Effect of carbon sources on Number & length of shoots in five different accessions of *Centella asiatica(L.)*



Graph-2: Effect of nitrogen sources on Number & length of shoots in five different accessions of *Centella asiatica(L.)*



Graph-3: Effect of different elicitors on Number & length of shoots in five different accessions of *Centella asiatica(L.)*

2) Estimation of asiaticoside by HPLC

The above mention treatment with carbon source, nitrogen source and elicitors shows different growth pattern in different accession. Among the five different accession 347492 shows the best growth in MS media containing malt extract (1mg/L) and supplemented with BAP (1.5mg/L). So the presence of asiaticoside as well as the relative percentage in malt extract treated in comparison to without treated is evaluate by reverse phase high performance liquid chromatography (RPHPLC) analysis of the methanolic extract of the leaf sample of *Centella asiatica* accession no 347492.

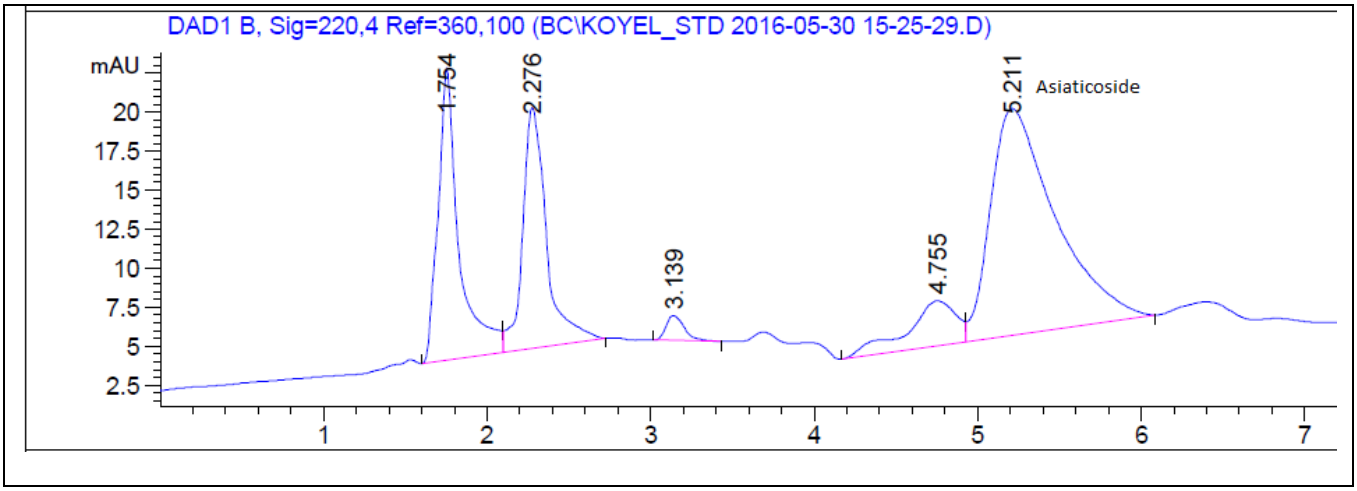


Figure-4: ***Chromatogram of standard asiaticoside from Centella asciatica(L.)***

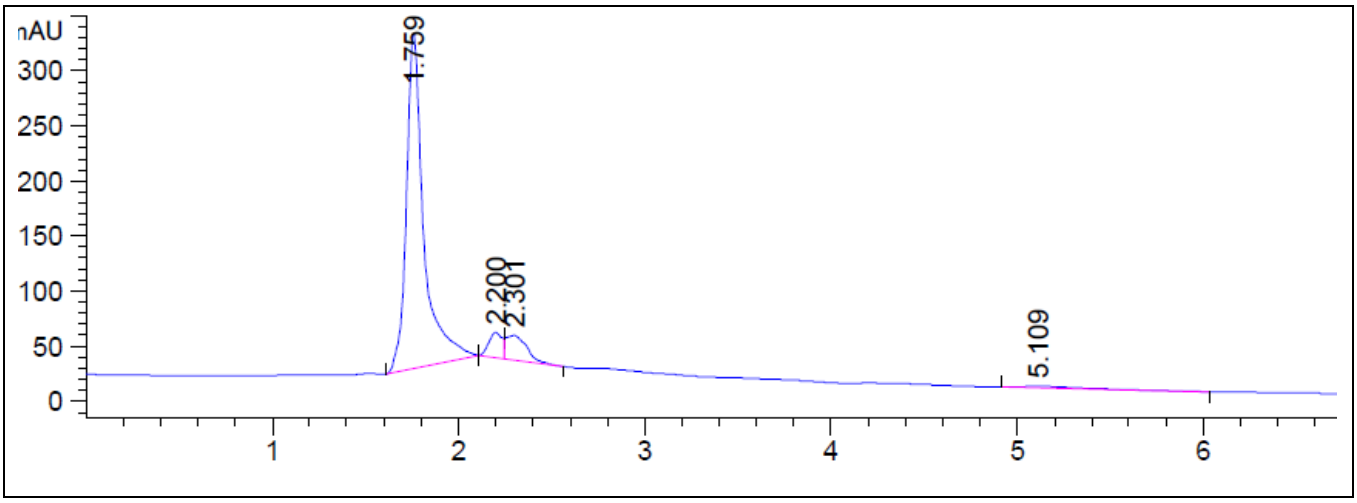


Figure-5: ***Chromatogram of Centella asciatica leaf extract grown in MS media.***

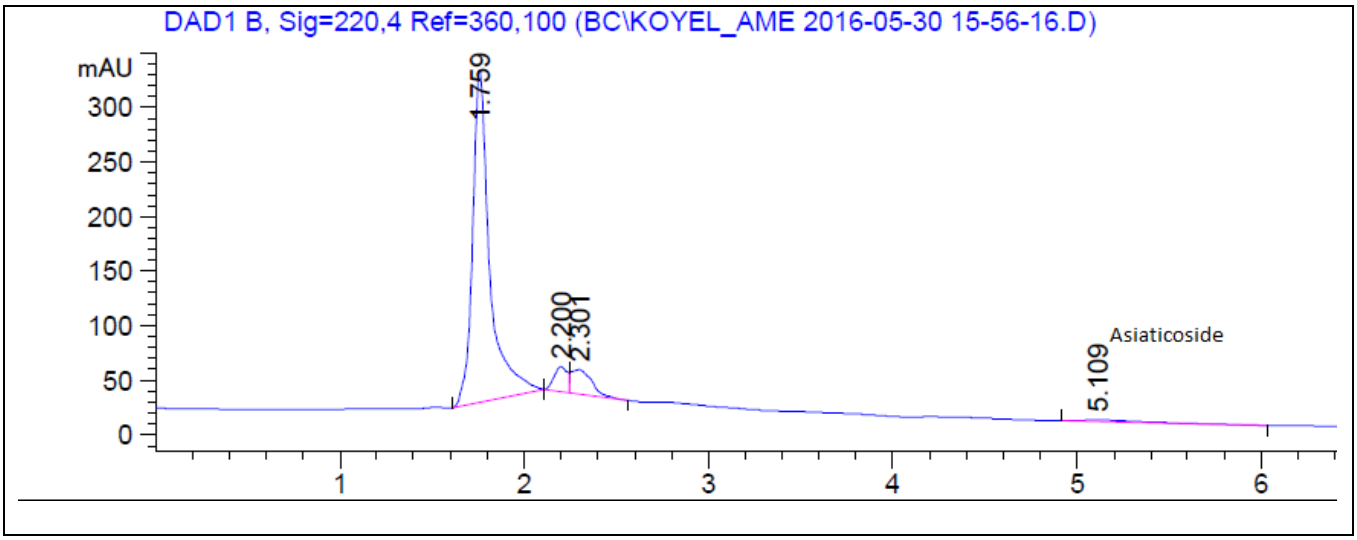


Figure-6: ***Chromatogram of Centella asciatica leaf extract grown in MS media supplemented with Malt Extract.***

Figure-4-6: Chromatogram of (4) standard asiaticoside, (5) extract analysed from plant grown in MS media without plant elicitor and (6) extract analysed from plant grown in MS media supplemented with malt extract as plant elicitor. Peaks of asiaticoside were found at 5.211, 5.109, 5.109 minutes in standard, without treated plant and treated plant respectively

Calculation from the HPLC chromatogram data

Concentration of stock asiaticoside = 100µg/ml

1µl stock sample contain 0.1 µg of asiaticoside.

5 µl stock sample contain 0.5µg of asiaticoside.

15 µl stock sample contain 1.5µg of asiaticoside.

Relative area percentage of standard asiaticoside = 53%

Relative area percentage of basal plant sample =0.692%

Relative area percentages of plant samples treated with malt extract are 1.396 %, 1.4%, 4.068%.

Formula:

Concentration of sample = (% area of sample × standard concentration)/ % area of standard

By applying the above mention formula concentration of asiaticoside in basal and treated plant samples were measured.

Figure 4 shows the chromatogram of standard asiaticoside and the retention time of asiaticoside in 5.2 minutes. Based on the two chromatogram (figure 5 and 6) data we can calculate that methanolic extract of malt extract treated sample (figure 6) contain 8.66 µg asiaticoside per gram of sample. Without treated sample (figure 5) contain 0.26 µg asiaticoside per gram of leaf sample. The above data clearly reveal that due to the treatment of plant with the elicitor Malt extract, it enhances the asiaticoside approximately 33 times higher.

Kim et al., 2004 also reported the enhanced production of asiaticoside by the use of elicitors such as methyl jasmonate. They reported 116.8 mg/l production of asiaticoside with the use of 0.1 mM MJ in B5 liquid media. They further increased the yield of asiaticoside by combining 0.1 mM methyl jasmonate with 0.025 mg/l TDZ [thidiazuron, 1-phenyl-3-(1,2,3-thidiazol-5-yl) urea] and achieved a production of 342.72mg/l. Kim et al., 2007 also reported use of methyl jasmonate in the increment of asiaticoside production in hairy root culture of *Centella asiatica*. Satheesan et al. 2012 also reported two-fold increase in the asiaticoside production by root colonization of a fungus named *Piriformospora indica*. Fungal mediated elicitation of asiaticoside was also reported by Prasad et al., 2013. Nutrient mediated increment in the asiaticoside production along with high yield of biomass was reported by Prasad et al., 2012 wherein they found this increment due to reduction in the total nitrogen concentration and copper starvation.

3) 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 accession of *Centella asiatica.* The plant sample contains fairly good fatty acid content with pentadecanoic acid hexadecanoic acid being present in significant amounts. Heptadecanoic acid, Stearic acid and octadecanoic acid were also obtained in moderate amounts in all five accessions.

Area Percent Report

Data Path : D:\MassHunter\GCMS\1\data\DTUIBT\17032016\
Data File : ca281374.D
Acq On : 17 Mar 2016 17:12
Operator :
Sample : ca281374
Misc : Acidic method
ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Wax_MS_FID_E5.M
Title :

Signal : TIC: ca281374.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.171	11	15	22	PB	359104	3857695	1.96%	0.470%
2	4.795	112	124	134	BB	233271	3397125	1.73%	0.414%
3	5.215	186	197	202	BV	386865	4396474	2.24%	0.536%
4	5.500	244	247	251	VV	265872	3010429	1.53%	0.367%
5	5.555	251	257	261	PV	513676	5609023	2.85%	0.683%

6	5.695	277	281	286	BV	2	464370	5797435	2.95%	0.706%
7	5.755	286	292	297	VV		2490858	28155849	14.32%	3.430%
8	5.892	310	315	329	PB		869908	10743398	5.46%	1.309%
9	6.127	349	357	364	PV		4492461	51040988	25.96%	6.218%
10	6.268	376	381	384	VV		445123	5218300	2.65%	0.636%
11	6.309	384	388	394	VV		9727130	112644953	57.28%	13.723%
12	6.360	394	397	404	VV		260566	3362402	1.71%	0.410%
13	6.568	427	434	440	VV		5849243	68488382	34.83%	8.344%
14	6.716	450	459	465	VV	2	274349	4201288	2.14%	0.512%
15	6.994	501	508	513	PV		2563129	29590612	15.05%	3.605%
16	9.040	859	866	877	VV		1708067	28353420	14.42%	3.454%
17	11.847	1333	1356	1367	BV		6589691	142073296	72.25%	17.309%
18	11.952	1367	1374	1389	VB		225491	5887760	2.99%	0.717%
19	14.564	1803	1831	1845	BB		571814	15644078	7.96%	1.906%
20	14.929	1873	1895	1907	BV	2	514932	13913873	7.08%	1.695%
21	15.715	2004	2032	2055	BV		6452642	196645807	100.00%	23.957%
22	16.849	2215	2230	2242	PV	2	2557870	78787518	40.07%	9.599%

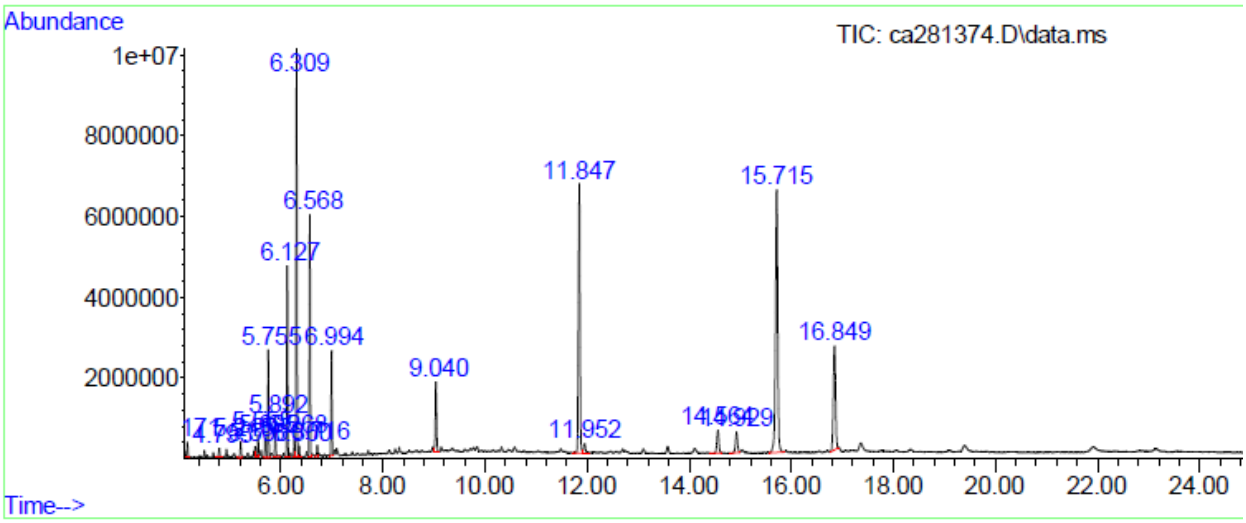


Figure 7: Chromatogram of *Centalla asiatica* accession no - 281374.

Data Path : D:\MassHunter\GCMS\1\data\DTUIBT\17032016\
Data File : ca331514.D
Acq On : 17 Mar 2016 15:49
Operator :
Sample : ca331514
Misc : Acidic method
ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Wax_MS_FID_E5.M
Title :

Signal : TIC: ca331514.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.163	9	13	22	PB	704699	7666021	3.99%	0.780%
2	4.496	63	72	76	BV	325707	3571578	1.86%	0.363%
3	4.672	92	102	111	BV	237690	2749318	1.43%	0.280%
4	4.788	111	122	133	PV	476813	6871605	3.57%	0.699%
5	4.931	133	148	152	PV 2	410495	5718143	2.97%	0.582%
6	5.208	185	196	201	BV	787018	8759421	4.56%	0.891%
7	5.348	210	220	228	BV 2	225528	3938391	2.05%	0.401%
8	5.470	237	242	243	VV	327899	3371852	1.75%	0.343%
9	5.493	243	246	250	VV	557050	6535115	3.40%	0.665%
10	5.549	250	255	260	VV	1057772	12703474	6.61%	1.292%
11	5.607	260	266	270	VV	350307	5589975	2.91%	0.569%
12	5.688	275	280	285	VV 2	930827	11533800	6.00%	1.173%
13	5.749	285	290	296	VV	4657241	53793672	27.98%	5.472%
14	5.817	296	302	309	VV 3	220320	4651884	2.42%	0.473%
15	5.886	309	314	329	PV	1719596	22121974	11.51%	2.250%

16	6.122	348	356	363	VV		8055674	94254276	49.03%	9.587%
17	6.263	375	380	383	BV		814239	9310296	4.84%	0.947%
18	6.305	383	388	393	VV		15796340	192254138	100.00%	19.555%
19	6.355	393	396	403	VV		287432	3738001	1.94%	0.380%
20	6.500	411	422	426	BV	2	234193	3040849	1.58%	0.309%
21	6.564	426	433	439	VV		5229896	61386771	31.93%	6.244%
22	6.711	454	459	465	VV	3	507028	6400813	3.33%	0.651%
23	6.990	501	507	513	BV		3497190	41041516	21.35%	4.175%
24	7.051	513	518	522	VV	2	211245	3152712	1.64%	0.321%
25	7.100	522	527	536	VV	4	218213	5121494	2.66%	0.521%
26	8.314	731	739	752	VV		217983	3865027	2.01%	0.393%
27	9.035	859	865	874	VV		834253	14333588	7.46%	1.458%
28	11.838	1331	1355	1368	BV		5913449	123321612	64.15%	12.544%
29	14.551	1807	1829	1842	BB		562162	15551570	8.09%	1.582%
30	14.918	1873	1893	1904	BV	3	392133	11260195	5.86%	1.145%
31	15.705	2002	2030	2058	BV	2	6061906	190088369	98.87%	19.335%
32	16.836	2209	2228	2240	BV		1518854	45450238	23.64%	4.623%

Sum of corrected areas: 983147689

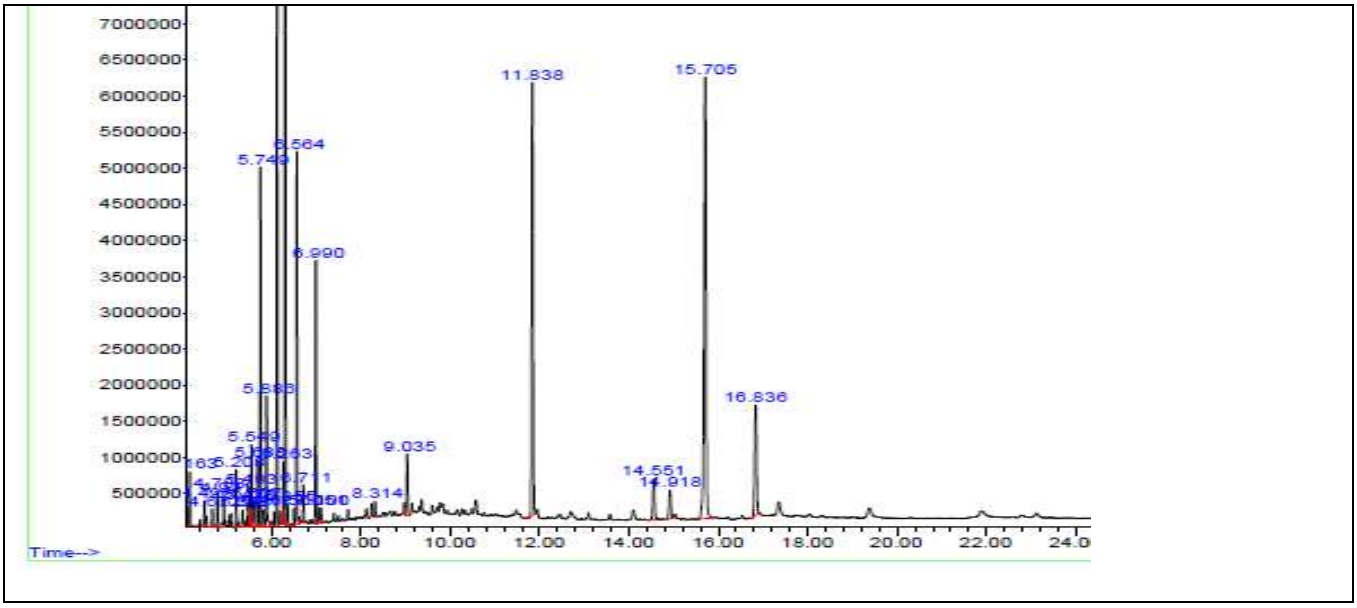


Figure 8: Chromatogram of *Centella asiatica*. Accession no - 331514.

Data Path : D:\MassHunter\GCMS\1\data\DTUIBT\17032016\
Data File : ca342109.D
Acq On : 17 Mar 2016 16:30
Operator :
Sample : ca342109
Misc : Acidic method
ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Wax_MS_FID_E5.M
Title :

Signal : TIC: ca342109.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.169	10	14	23	PV	482374	4901175	2.89%	0.606%
2	4.502	63	73	76	BV	215676	2215204	1.31%	0.274%
3	4.793	112	123	133	BV	295719	4256527	2.51%	0.526%
4	4.936	133	148	152	PV 2	257199	3655237	2.16%	0.452%
5	5.212	185	197	201	BV	489918	5622806	3.32%	0.695%
6	5.497	244	246	250	VV	330593	3907359	2.31%	0.483%
7	5.552	250	256	260	VV	637978	7837133	4.63%	0.969%
8	5.651	270	273	277	VV	527661	5904024	3.48%	0.730%
9	5.691	277	280	286	VV 2	558367	6989274	4.12%	0.864%
10	5.751	286	291	296	VV	2848324	32708165	19.30%	4.046%

11	5.889	310	315	328	PB	1016246	12050871	7.11%	1.491%
12	6.124	348	356	363	VV	4940505	58185702	34.34%	7.197%
13	6.265	376	381	383	PV	499124	5574778	3.29%	0.690%
14	6.306	383	388	393	VV	10759513	125280033	73.94%	15.496%
15	6.565	426	433	439	VV	3265493	38008743	22.43%	4.701%
16	6.713	449	459	466	VV 3	328017	5479986	3.23%	0.678%
17	6.991	501	508	513	VV	2302773	27547863	16.26%	3.407%
18	7.091	521	525	536	VV 3	224791	4779470	2.82%	0.591%
19	9.038	857	865	877	VV	4074845	68062196	40.17%	8.419%
20	11.840	1344	1355	1367	BV	5618183	121878011	71.93%	15.075%
21	11.946	1367	1374	1387	VB	247928	6085282	3.59%	0.753%
22	12.693	1490	1504	1531	VB 7	218088	7575511	4.47%	0.937%
23	14.556	1809	1830	1843	BB 2	555526	15379983	9.08%	1.902%
24	14.921	1855	1893	1905	BV 3	490298	13352013	7.88%	1.651%
25	15.708	2006	2031	2056	BV	5596638	169437993	100.00%	20.958%
26	16.837	2209	2228	2249	BB 2	1642287	51802699	30.57%	6.407%

Sum of corrected areas: 808478036

Wax_MS_FID_E5.M Thu Mar 17 18:29:21 2016

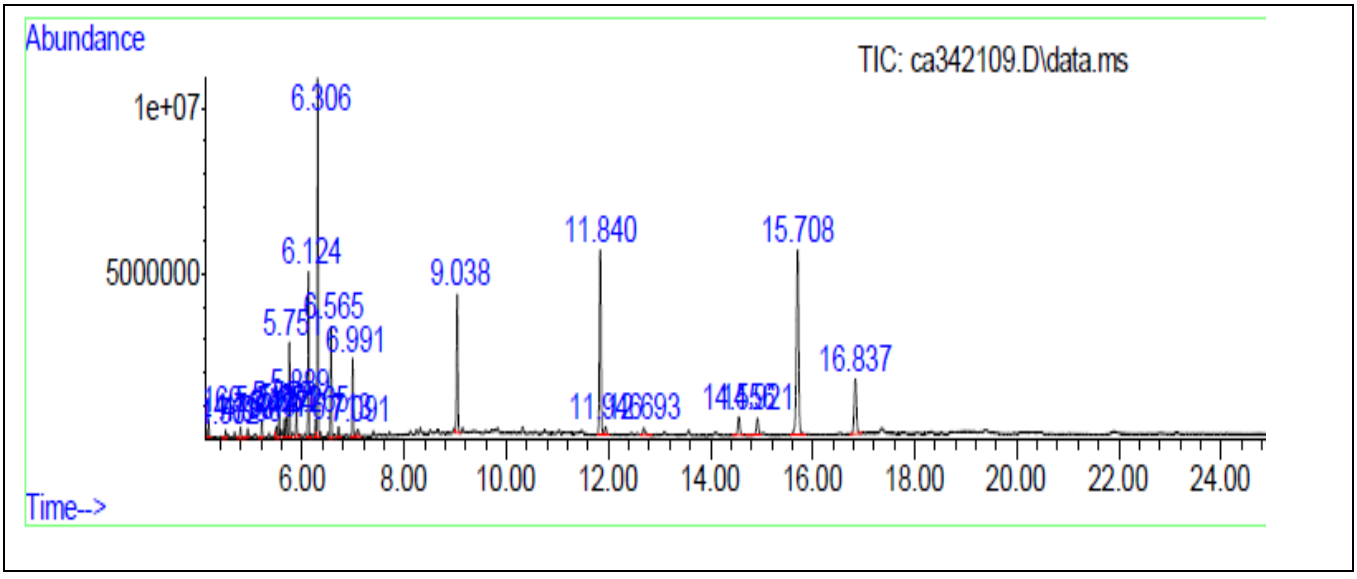


Figure 9: Chromatogram of *Centalla asiatica*. Accession no - 342109.

Data Path : D:\MassHunter\GCMS\1\data\DTUIBT\17032016\
Data File : ca347492.D
Acq On : 17 Mar 2016 17:53
Operator :
Sample : ca347492
Misc : Acidic method
ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Wax_MS_FID_E5.M
Title :

Signal : TIC: ca347492.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.135	3	8	13	BV 2	617569	7498188	1.40%	0.260%
2	4.182	13	17	25	VB	1269736	13992161	2.61%	0.486%
3	4.513	68	75	79	PV	623238	7281338	1.36%	0.253%
4	4.559	79	83	87	VV	263026	3051107	0.57%	0.106%
5	4.688	100	105	113	BV	431098	5161817	0.96%	0.179%
6	4.803	113	125	135	VV	860436	12225089	2.28%	0.425%
7	4.946	144	150	154	VV 2	737311	9263734	1.73%	0.322%
8	5.076	169	173	174	PV	255314	2423843	0.45%	0.084%
9	5.097	174	177	180	VV	305536	3331293	0.62%	0.116%
10	5.222	188	198	203	BV	1453361	16148936	3.01%	0.561%
11	5.362	216	223	230	PV 2	416645	7489016	1.40%	0.260%
12	5.484	240	244	246	VV	592478	6190270	1.16%	0.215%
13	5.507	246	248	252	VV	992362	11617423	2.17%	0.404%
14	5.563	252	258	262	VV	1873989	22715754	4.24%	0.789%
15	5.620	262	268	272	VV	647206	10386123	1.94%	0.361%
16	5.702	278	282	288	VV 2	1602862	20453907	3.82%	0.711%
17	5.762	288	293	298	VV	7657876	89780424	16.76%	3.119%
18	5.827	298	304	311	VV 4	388189	8333963	1.56%	0.290%
19	5.899	311	317	329	VV	2981568	38853709	7.25%	1.350%
20	6.066	340	346	350	VV 3	337494	4875069	0.91%	0.169%

21	6.136	350	358	365	VV		12547557	151100271	28.20%	5.249%
22	6.201	365	370	378	VV	3	351170	6695869	1.25%	0.233%
23	6.276	378	383	385	VV		1409974	16799016	3.14%	0.584%
24	6.320	385	390	395	VV		21354469	285733720	53.33%	9.927%
25	6.366	395	398	404	VV		701959	9142968	1.71%	0.318%
26	6.512	413	424	428	PV		408970	5749137	1.07%	0.200%
27	6.573	428	435	440	VV		3198931	38938101	7.27%	1.353%
28	6.723	456	461	480	VV	3	950757	18085942	3.38%	0.628%
29	6.863	480	485	490	VV		572395	7555512	1.41%	0.262%
30	7.005	502	510	515	VV		17505840	233717038	43.62%	8.119%
31	7.060	515	520	524	VV	2	512790	9164866	1.71%	0.318%
32	7.113	524	529	538	VV	3	353924	8857156	1.65%	0.308%
33	7.410	569	581	585	VV	3	238162	5205964	0.97%	0.181%
34	7.721	624	635	638	VV		330483	5458640	1.02%	0.190%
35	8.135	702	707	716	VV	7	284977	7872492	1.47%	0.273%

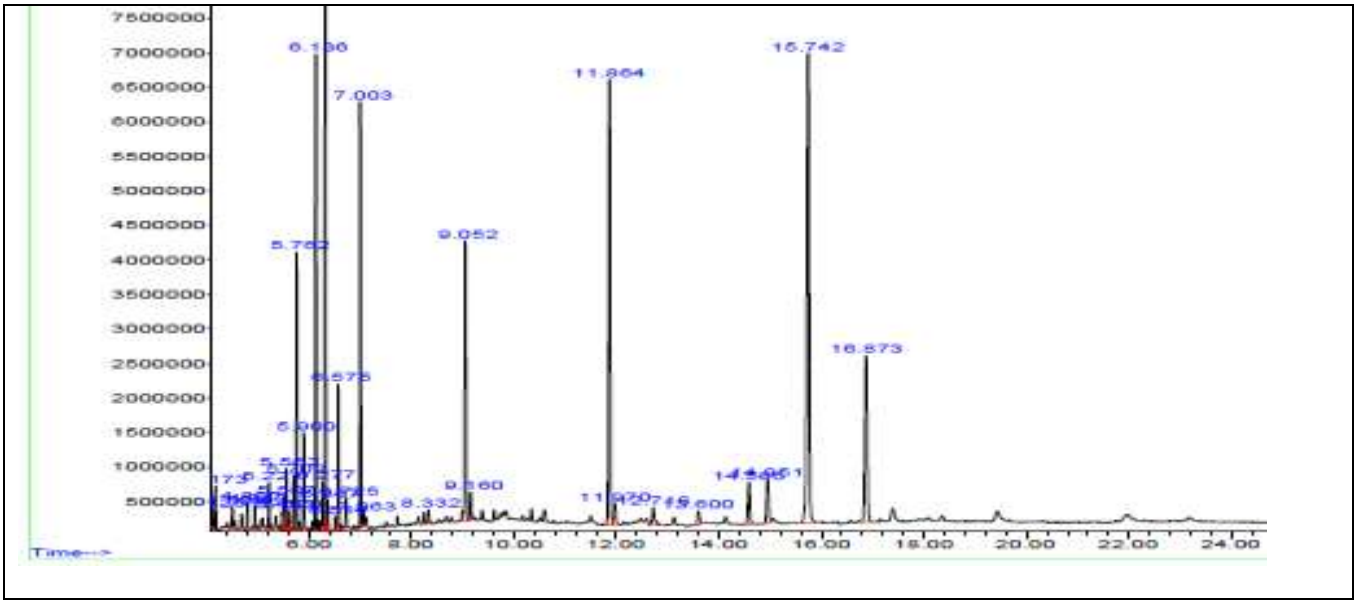


Figure 10: Chromatogram of *Centalla asiatica*. Accession no - 347492.

Area Percent Report

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Data File : ca383913.D
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Sample : ca383913
Misc : Acidic method
ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\MassHunter\GCMS\1\methods\Wax_MS_FID_E5.M
Title :

Signal : TIC: ca383913.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.126	3	7	11	BV 2	274437	3271376	1.49%	0.308%
2	4.173	11	15	20	PV	609633	6347488	2.88%	0.598%
3	4.508	66	74	78	PV	300649	3565204	1.62%	0.336%
4	4.800	114	125	133	PB	384531	5527425	2.51%	0.520%
5	4.943	144	150	154	VV	332810	4122242	1.87%	0.388%
6	5.221	188	198	203	BV	656051	7291513	3.31%	0.687%
7	5.483	240	244	246	PV	268322	2715275	1.23%	0.256%
8	5.507	246	248	252	VV	472289	5531208	2.51%	0.521%
9	5.562	252	258	262	VV	890933	10536974	4.79%	0.992%
10	5.620	262	268	278	VV	288695	4631809	2.11%	0.436%
11	5.702	278	282	288	PV 2	774841	9493438	4.31%	0.894%
12	5.762	288	293	298	VV	3998541	45155660	20.52%	4.252%
13	5.900	311	317	329	PV	1426326	17580924	7.99%	1.655%
14	6.136	350	358	365	VV	6867652	80493870	36.58%	7.579%
15	6.277	377	383	385	VV	715265	8331591	3.79%	0.785%
16	6.318	385	390	395	VV	13967515	169777160	77.16%	15.986%
17	6.367	395	399	405	VV	450501	5927571	2.69%	0.558%
18	6.514	414	424	428	PV 2	201212	2503592	1.14%	0.236%
19	6.575	428	435	441	VV	2127430	25358864	11.53%	2.388%
20	6.725	451	461	468	VV 2	433846	5739063	2.61%	0.540%

21	7.003	498	510	515	PV	5879340	71194523	32.36%	6.704%
22	7.063	515	520	524	PV 2	195008	2754482	1.25%	0.259%
23	8.332	733	742	756	VB	206719	3847530	1.75%	0.362%
24	9.052	861	868	879	VV	4012565	66665800	30.30%	6.277%
25	9.160	879	887	894	PV	397103	6075166	2.76%	0.572%
26	11.864	1341	1359	1371	BV	6346477	135236173	61.46%	12.734%
27	11.970	1371	1378	1391	VB	312049	7558199	3.44%	0.712%
28	12.718	1494	1508	1534	VB 8	250495	8479212	3.85%	0.798%
29	13.600	1650	1663	1680	BV 3	198090	5831398	2.65%	0.549%
30	14.586	1815	1835	1851	BB 2	606835	17165084	7.80%	1.616%
31	14.951	1880	1899	1910	BV 4	624296	17736618	8.06%	1.670%
32	15.742	2008	2037	2061	BB	6808215	220030863	100.00%	20.718%
33	16.873	2217	2234	2257	BB	2397553	75527007	34.33%	7.112%

Sum of corrected areas: 1062004303

Wax_MS_FID_E5.M Thu Mar 17 19:11:30 2016

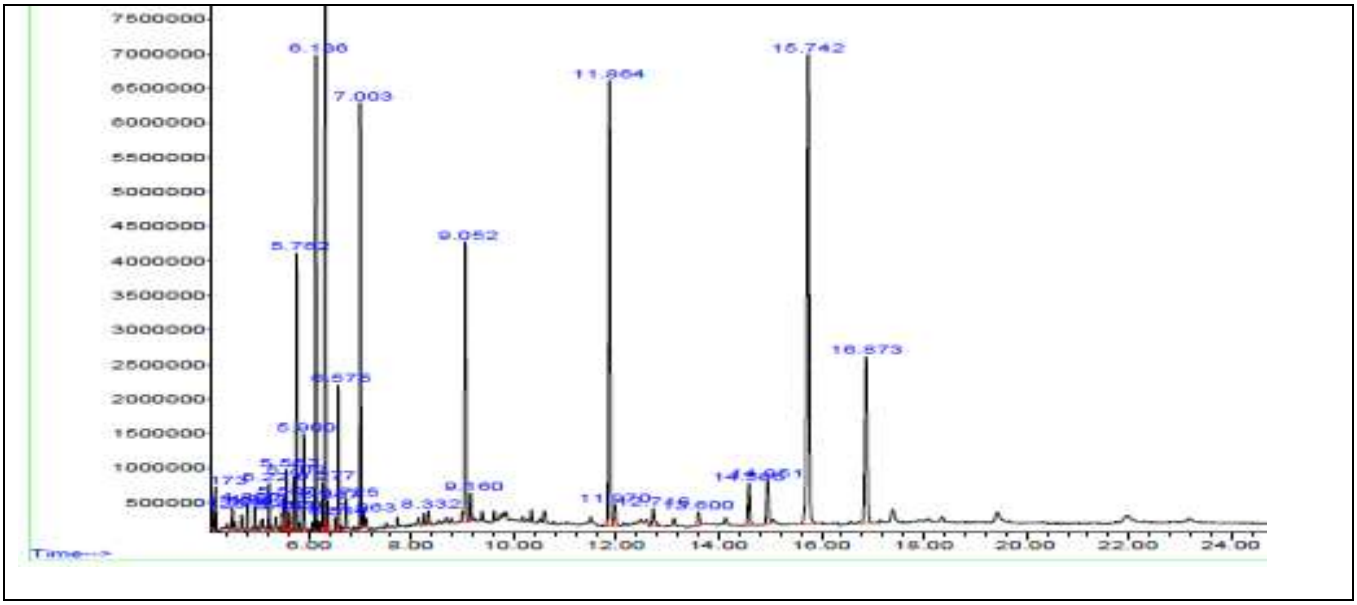

Figure 11: Chromatogram of *Centella asiatica*. Accession no - 383913.

Fatty Acid	Relative %age Content of Fatty acid				
	Accession No. 281374	Accession No. 383913	Accession No. 342109	Accession No. 347492	Accession No. 331514
Pentadecanoic acid	17.31%	12.734	15.08%	13.65%	12.54%
Hexadecanoic acid	1.91%	1.62%	1.90%	1.96%	1.58%
Octadecanoic acid	1.70%	1.67%	1.65%	1.68%	1.15%
9,12 Octadecadienoic acid	23.957	20.72%	20.96%	18.62%	19.34%
9,12,15 Octadecatrienoic acid	9.599	7.11%	6.41%	9.98%	4.63%

Table 4: Percentage of methylated fatty acids of five different accession of *centella asiatica*.

The analysis of fatty acid from five different accession of *centella asiatica* by GC-MS showed that *Centella asiatica* is a rich source of both saturated and unsaturated fatty acids. Chromatogram data shows that the plant contain various bioactive constituents including pentadecanoic acid, octadecanoic acid, octadecadienoic acid, octadecatrienoic acid, stearic acid in major concentration compared to hexadecanoic acid and heptadecanoicacid which are present in very less amount in these five different accession if *C. asiatica.*

Saturated fatty acids

The above chromatogram data reveal that the plant contains pentadecanoic acids (C15) [12.54% to 17.31%], hexadecanoic acids C16 [1.58% to 1.96%], and octadecanoic acids C18 [1.15% to 1.70%] of relative percentage areas.

Unsatutated fatty acids

The above chromatogram data reveal that the plant contains 9,12 octadecadiinoic acids (C18:2)[18.62% to 23.96%] and 9, 12, 15 octadecatrienoic acids (C18:3) [1.15% to 1.70%] of relative percentage areas.

Unsaturated fatty acids, Octadecadienoic acids and Octadecatrienoic are the most important essential fatty acids as because our body cannot synthesis these fatty acids. Common name of these fatty acids are linoleic acid and linolenic acid respectively which are essential for fatty acids. Linoleic acid is important for growth and is very potent prostaglandin biosynthesis inhebitors. Palmitic acid (C16) reduces the risk of cardiovascular disease. Stearic acid used in baked food items.

So this plant has very good industrial value. It is also used in cholesterol-lowering diets. These potential accessions can be utilized for the high yield of fatty acid production. Very less work has been reported related to the FAME profile of *Centella asiatica* which provided an opportunity to carry out this investigation. Jahan et al., 2015 conducted a study related to

elemental as well as fatty acid content of four medicinally important plant i.e. *Kaiempferia rotunda, Cuscuta reflexa, Centella asiatica* and *Asparagus racemosus*. Within *Centella asiatica*, they found Hexadecanoic acid (9.96%), Heptadecanoic acid (3.28%) and Octadecanoic acid (8.34%) but they also reported no presence of 9,12-Octadecadienoic acid. Although in our study [18.62% to 23.96%] of 9,12-Octadecadienoic acid has been investigated.

CONCLUSION

From the above mention studies it can be concluded that different carbon sources, nitrogen sources and elicitors act on the metabolic activity of the plant and trigger the shoot multiplication along with enhancement of plant's active compounds. This study concluded that the sucrose showed better carbon source than fructose, ammonium nitrate is potential source of nitrogen for the proliferation of shoots in *Centella asiatica* in the capability to rapid multiplication of the plant. Malt extract performed on metabolic pathways, showed the highest number of shoot proliferation and it facilitated asiaticoside production. The plant reveals diverse pattern of growth among the five different accessions. The most potential accession depend on number of shoot proliferation, elicitated with malt extract in MS media shown the capacity to produce 33 times more asiaticoside production in comparison to the basal media grown plants. FAME analysis of *Centella asiatica* revealed that individual accessions have the potential to provide high yield of important fatty acids. These culture conditions and potential accessions can be used for high yield of biomass as well as asiaticoside and fatty acid production under lab conditions which may be beneficial for the fulfillment of their demand.

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