# EFFECT OF DIFFERENT GROWTH HORMONES AND IN-VITRO PROPAGATION OF STEVIA REBAUDIANA AND QUANTIFICATION OF SECONDARY METABOLITES

*Project report submitted in partial fulfillment of the requirement for the degree of*

**BACHELOR OF TECHNOLOGY**

**IN**

**BIOTECHNOLOGY**

By

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# CERTIFICATE

This is to certify that the work titled **"Effect of different growth hormones and invitro propagation of Stevia rebudiana and quantification of secondary metabolites"** pursued by **Anubhavi Singh** in partial fulfillment for the award of degree of **B.Tech in Biotechnology** from Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any another University or Institute for the award of any other degree, diploma or appreciation.

**Signature of Supervisor** -

**Name of Supervisor** - **Dr. Hemant Sood ( Associate Professor )**

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**Date** -

# DECLARATION

The report entitled, **"Effect of different growth hormones and invitro propagation of Stevia rebaudiana and quantification of secondary metabolites."** has been solely submitted to Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat in partial fulfillment for the award of degree of B.Tech in Biotechnology. The work has not been submitted to any other organization for any degree, diploma or appreciation.

The concept and hypothesis behind the research work and experiments were planned and executed under the guidance of my supervisor, **Dr. Hemant Sood** and hence, it's a work of complete originality. Wherever any experimental data or materials (data, analysis, figures, tables, texts, graphs) from other sources has been used, we have given due credit by citing them in text of the thesis.

**Signature of Student** :

**Name of Student** : Anubhavi Singh

**Enrollment No**. : 151845

**Date** :

# ACKNOWLEDGEMENT

The success of the project does not lies solely in its end product but the journey lived and the lessons learnt along the way. I extend my first and foremost gratitude to our "Department of Biotechnology and Bioinformatics" for the confidence bestowed upon me and entrusting my project entitled, "Effect of different growth hormones and invitro propagation of Stevia rebudiana and quantification of secondary metabolites."

At this juncture, with proud privilege and profound sense of gratitude I feel honoured in expressing our deepest appreciation to Dr. Hemant Sood for being a lot more than just our supervisor and going beyond the call of duty in our guidance, support, advice and motivation throughout. She has been the source of inspiration of come what may, these issues cannot bring you down. Sincere thanks for making the resources available at the right time and providing your valuable insights.

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Special thanks to my parents for their infinite patience and understanding and lastly but most importantly God, who in His mysterious ways, always made things work out in the end.

In gratitude,

Anubhavi Singh

# SUMMARY

*Stevia rebaudiana* being a natural sweetener, being utilized worldwide in food entities like green teas, syrups, ready to eat meals, etc. The traditional sugar we use in our daily lives can be substituted by Stevia as well.

In this study, we have utilized MS media of varied concentrations and growth hormones, which have enhanced the effect of Stevia, with respect to the composition of growth hormones for callus induction with KN and IBA their concentration being 1 :3 (mg/l) and BAP and NAA with a concentration of 0.5 : 2 (mg/l) ; these conditions have been optimized for shoot regeneration and multiplicity of In-vitro plantlets.

GC analysis was carried for both field grown leaves and in-vitro leaves which reported the presence Diterpene which is an alkaloid. Both sample types were detected with compounds containing Diterpene and Sesquiterpene properties. In one year old field grown shoots, 86 molecules are identified (Table 6), whereas in two month old tissue cultured shoots, 59 molecules were identified (Table 7) in which n-alkenes, n-alkanes, fatty acids, terpenes, diterpene, monoterpenes sesquiterpenes were identified.

HPLC was carried out for the estimation of steviol glycoside concentration in field grown tissue and in-vitro grown tissue. The concentration reported in field grown tissues was 0.5287% and in in-vitro grown tissues was 3.5932% for the first time.

We can conclude that these optimized conditions conducted in the laboratory for a period of 1 year, have high significance for commercial purposes.

# INTRODUCTION

Stevia rebaudiana originates from the sunflower family [1], it is also known as candy leaf or sweet leaf, and is a member of the family Composite and it is a native to Paraguay [2] [3] also, found in the region of South Africa. (Brazil and Paraguay) [4]

Stevia is an exotic herb that obtains a height of 30.5cm to 80 cm. These aromatic leaves are 2.5 cm long with a distinct midrib. The small tubular flowers have five white petals and are present in terminal clusters; the flowers are usually extracted from the main plant part to improve the flavor of the leaves. Germination from the seedling is difficult hence mostly they are grown from cuttings. The plant requires nutrient rich and well-drained soil which can thrive in warm and humid climates.

## Plant Morphology :

**Growth Form**: Herbaceous plant grows to a height of 1m. A constant in tropical and sub-tropical climates but grown in cooler climatic conditions as well.

**Foliage:** Leaves are oval and elliptical with minute serrated margins, it is edible in raw form as well, reported to be 25 - 30 times sweeter than table sugar, it is a source of stevioside and rebaudiosides compounds which is used in food and pharmaceutical industries.

**Flowers**: They are white, small and insignificant, which is formed in clusters of 2 -6 florets; it is a free-flowering plant.

**Fruits**: It has indehiscent properties and characteristics.

## Taxonomy:

The current taxonomic status of the genus Stevia is quite complex due to the generally similar morphology.

Classification of *S. Rebaudiana* is given below:

**Kingdom** Plantae

**Clade:** Angiosperms

**Clade**: Eudicots

**Clade**: Asteroids

**Order**: Asterales

**Family**: Asteraceae

**Genus**: Stevia

**Species:** S. rebaudiana

**Binomial name** Stevia rebaudiana

**Figure 1.** ***Stevia rebaudiana***

Stevia is widely known for its sweet leaves, which produce diterpene glycosides (stevioside and rebaudiosides) which have 250–300 times the sweetness of sugar. It's a medicinal herb, consisting non-caloric sweetening properties, it is an inevitable alternative to sugar as there are millions of diabetics across the globe [5].

To exploit industrial applications of stevia, its massive scale production is needed. Seed germination is a difficult task as they have a small endosperm and infertile seeds [16,17]. Certain plantlets produce infertile seeds due to incompatibility [19,18]. There are various reports showcasing the conventional propagation methods of Stevia through stem cuttings. The amount of stem cuttings would be voluminous which would be a setback in mass multiplication of plants is. As the origin of plant is diminishing, the conventional methodologies are not full filling for its production on a large scale in the market and fulfill the cane sugar demands around the globe.[20] We see stevia as an alternative to sugar in the

Foreseeable future that means almost 40,000 tones stevia is needed to replace artificial sugar and cane sugar through conventional methods but it is still quite impossible to meet the demand. Micro-propagation protocols of Stevia have been reported from different explants like a leaf [22, 23, 24], nodal [25, 27,] and shoot tip explants [33,34,35] but production of quality rich herbal source was not highlighted so the present investigation was emphasized on the development of an efficient, reproducible in vitro micro-propagation protocol for high frequency shoot multiplication by using different concentration and combination of the effect was observed in tissue culture and field grown shoots by carrying out GCMS analysis.

# REVIEW OF LITERATURE

Stevia leaves are being used by the Guarani people. Traditionally, this plant was being used to sweeten yerba maté and numerous teas; it has also been used in holistic medicines. The plant was initially discovered by a Swiss botanist in 1899 namely, Mosè Giacomo Bertoni (known in Spanish as Moisés Santiago Bertoni). He had announced this discovery of the sweet-tasting plant and had named it Eupatorium rebaudiana. Later with time progressing, Japanese scientists had developed a stevia-derived sweetener, which was a success among people. Despite a few restrictions on the plant due to carcinogenic contents, it was approved by the U.S. Food and Drug Administration (FDA) in 2008. The European Union later made stevia a viable product in 2011.

## Cultivation and propagation of Stevia rebaudiana:

It is grown in herbariums and pots in gardens. It tends to produce more flowers and seeds when kept in a sunlit environment. It prefers moist, well-drained and slightly acidic soils (pH 6.1 - 6.5). It can also grow in infertile sandy soils. Plant should be avoided from nitrogen based fertilizers. The sweetness content is higher if the plant is harvested in the morning. The most suitable way to propagate it is by stem cuttings. Black seeds of the plant are reported to germinate better than the smaller tan-colored seeds.

## Etymology:

The genus epithet 'Stevia' is named after 16th century Spanish botanist and physician Pedro Jaime Esteve. Species epithet 'rebaudiana' is named after 19th century Paraguayan chemist Oviodio Rebaudi, who had extracted sweet compounds from the plant after isolation for the first time.

## Phytochemistry of *Stevia rebaudiana* :

The compounds found in the leaves of Stevia rebaudiana are the sweet tasting diterpenoid glycosides. There are two main glycosides which are of significance to the commercial market, are Stevioside and Rebaudioside A. The other compounds which are of importance include Rebaudioside B to E, Dulcoside A and C. The diterpene glycosides from S. rebaudiana contain a glycoside called steviol (13hydroxy-ent-kaur-16-en-19-oic acid), which differs only in the glycoside constituents that is attached at C-13 and/or C-19.

Stevioside is a core compound in the plant stevia. Its percentage ranges from 5 to 10 percent. Rebaudioside A is the second most common compound that exhibits the sweet taste in stevia, it ranges from 2 to 3 percent. It is reported to have a more pleasant taste than stevioside and is even more water soluble. Rebaudioside B, D, and E might be present in lesser quantities. The two main compounds present in stevia i.e. stevioside and rebaudioside, they were first isolated by two French chemists, Bridel and Lavielle (1931).

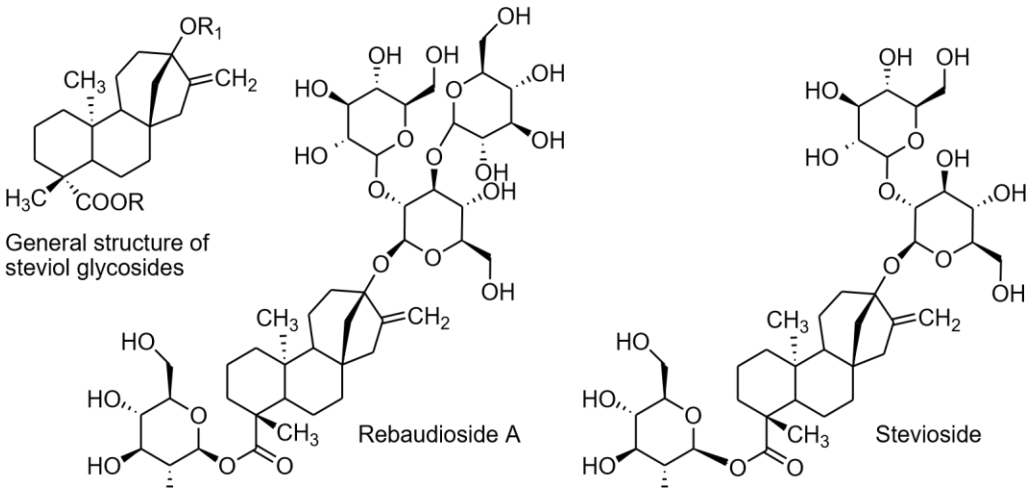


**Figure 2.**General structure of steviol glycoside and representative structure of rebaudioside A and Stevioside.

## Pharmacological properties of *Stevia rebaudiana* :

A sugar substitute that is used in food entities to enhance the sweet effect in terms of taste. There are various substitutes of sugar available, which could be natural and synthetic. Food additives which are commercially being produced, should be approved by the FDA, and that additive should be categorized under Generally Recognized as Safe (GRAS) list of additives. These substitutes are used by globally for achieving purposes like weight loss, dental care, diabetes and hypoglycemia. The main substitutes available for human use are : aspartame, cyclamate, saccharin and sucralose. Aspartame, was produced from two amino acids namely aspartic acid and phenylalanine. It is about 200 times sweeter than sucrose. The safety and ethical use of aspartame has been studied and experimented extensively on animal studies, clinical and epidemiological research. Aspartame is known to be rapidly hydrolyzed in the small intestines. Additionally, people suffering from the genetic disorder called phenylketonuria should avoid aspartame as they have a reduced ability to metabolize the naturally occurring essential amino acid called phenylalanine. The admissible daily intake value for aspartame is calculated to be 40 mg/kg of the body weight.

## Beneficial effects on human health :

*Stevia rebaudiana* extracts have suggested to have beneficial effects on human health abnormalities like antihypertensive, anti-hyperglycemic, anti-carcinogenic and anti-human rotavirus activities. It also affects the metabolism of glucose and renal function. The requirement of essential oils in our daily life has increased the probability of the usage of stevia extracts in various domains of human life like pharmacy, cosmetics and food and beverage industries. This has made a remarkable research domain available.

Stevia is nutrient-rich in nature. It contains substantial amounts of protein, calcium, phosphorous and few other nutrients. Stevia being a substitute of sugar, its taste has a slower onset and a longer duration than sugar. Some of its extracts might have a bitter or licorice-like aftertaste. The sweet compounds pass through the digestive process of the body without chemically breaking down, thereby making it a safe food substance for human-use. Furthermore, it is a plethora of distinction having commercial and therapeutic value.

## GC Analysis :

In recent years, GC-MS has emerged as one of the important techniques in natural products research. Gas chromatography provides a prominent resolution of the components present in a mixture. Identification of the separated compounds can be achieved through their characteristic molecular mass spectra. GC-MS with database search facility and Relative retention indices (RRI) data are very powerful techniques to identify the volatile constituents in herbal drug.

## Biotechnological approach on *Stevia rebaudiana* :

The development of methodologies that utilize biotechnology like plant tissue culture, micropropagation, root culture and transformed root culture, they represent an alternative approach for the search of secondary metabolites and they allow genetic stability to occur. Thus, with the development of rapid root growth, it would be possible to obtain sufficient material for the production of extracts for the use of commercial production of compounds of interest.

Considering the results from previous studies on *Stevia rebaudiana* that has been reported by numerous research groups S. rebaudiana in the form of in-vitro represent a biotechnological alternative for obtaining the metabolites.

## **HPLC Analysis :** .

The applications of high performance liquid chromatography in food entities and technology involves an analytical and quantitative testing of the products like plant extracts and gives an assurance of product quality with increased productivity and concentration. It is used for the analysis of components in raw and processed products. [45]

## 7: AIMS AND OBJECTIVES:

Thus taking into consideration high demand of *Stevia* raw material worldwide, occurrence of species in Paraguay and their inherent difficulties in cultivation, over exploitation from native population, this study has been proposed to investigate the possibilities to enhance the production of bioactive compounds stevioside and rebaudiosides. The proposed study has following objectives:

**Objectives :**

- To study the effect of growth hormones in callus induction of *Stevia rebudiana.*
- To study direct and indirect organogenesis responses in *Stevia rebudiana*.
- Quantification of secondary metabolite in *Stevia rebaudiana*

## 8: METHODS AND MATERIAL:

### Selection of plant material :

Six month S*tevia rebaudiana* plants were procured from the nursery and authenticated from UHF, Nauni having UHF-Herbarium number 13585 and maintained in the glass-house of Jaypee University of Information Technology, Waknaghat, H.P., and India under natural light conditions.

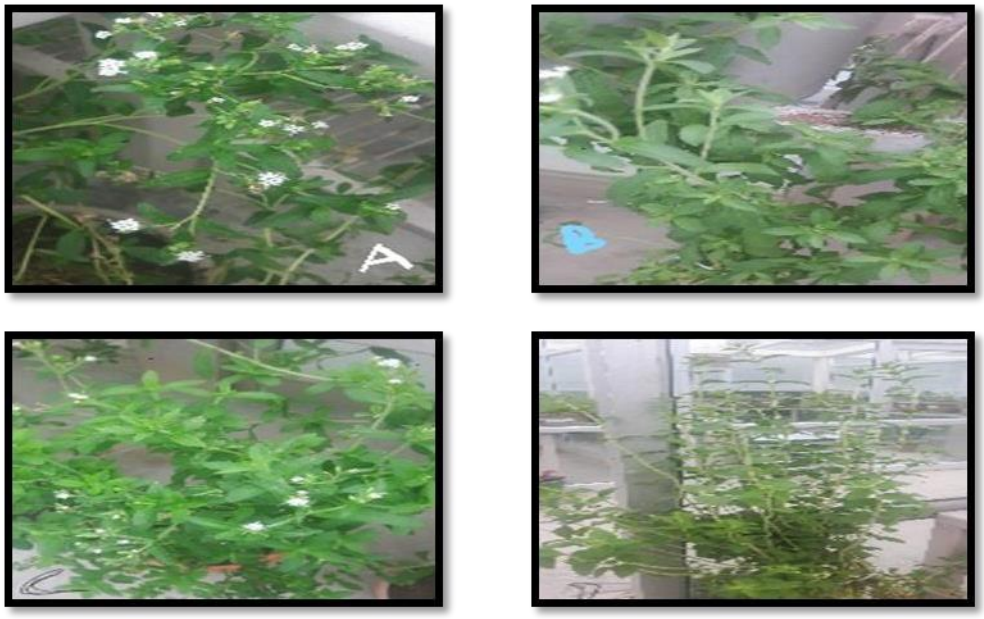

**Figure 2 : Four different strains of *Stevia rebaudiana***

### Media Preparation and Culture Conditions :

MS media (Murashige and Skoog 1962) supplemented with different concentration of growth hormones have been tested for establishment of a culture (Table 1), sucrose (30 g/L) and agar (9 g/L) as a gelling agent. The pH was adjusted to 5.7 using 0.1 N HCl and 0.1 N NaOH and 50 ml of media was dispensed in each jar prior to autoclaving at 121 C, 15 lb. inch-2 pressure for 20 min. The cultures were kept at 25 ± $2^0$C, with 70 % relative humidity, 16 h day/8 h night photoperiod at a photosynthetic photon flux density of 40 l mol m-2 s-1 provided by cool white fluorescent tubes (Philips, India) with sub culturing after every 4 weeks in a plant tissue culture chamber.

**Table 1: MS media supplemented with different concentrations of growth hormones for callus induction in *Stevia rebudiana. (*For 4 different strains of *Rebaudiana)***

| MS medium label | IBA (mg/l) | TDZ (mg/L) | KN (mg/L) | IAA (mg/L) | BAP (mg/L) | 2,4, D (mg/L) | NAA (mg/L) |
|---|---|---|---|---|---|---|---|
| CS1 | 3 | | 1 | | | | |
| CS2 | 1 | | 3 | | | | |
| CS3 | | | 1 | 3 | | | |
| CS4 | | | | | 0.5 | | 2 |
| CS5 | 0.5 | | | | | 2 | 0.5 |
| CS6 | 0.5 | 1 | | | | | |
| CS7 | 1 | 1.5 | | | | | |
| CS8 | 1.5 | | 3 | | 1 | | |
| CS9 | 3 | | 1 | | | | |
| CS10 | | | | | 1 | | 2 |
| CS11 | 0.5 | | | | | | 2 |

**Table 2: MS media supplemented with different concentrations of growth hormones for Direct Organogenesis in *Stevia Rebaudiana (*for four different strains of *S. rebaudiana)***

| ***MS Media label*** | ***IBA(mg/l)*** | ***IAA(mg/l)*** | ***Kinetin(mg/l)*** | ***BAP(mg/l)*** | ***2,4-D(mg/l)*** | ***NAA(mg/l)*** | ***TDZ(mg/l)*** |
|---|---|---|---|---|---|---|---|
| ***DO1*** | ***1*** | | ***0.5*** | | | | |
| ***DO2*** | ***2*** | | ***1*** | | | | |
| ***DO3*** | ***3*** | | ***1*** | | | | |
| ***DO4*** | | ***2*** | ***1*** | | | | |
| ***DO5*** | | ***3*** | ***1*** | | | | |
| ***DO6*** | | | | ***0.5*** | | ***1*** | |
| ***DO7*** | | | | | ***1*** | | |
| ***DO8*** | | | | ***1*** | | ***2*** | |
| ***DO9*** | | | ***1*** | | | | ***2*** |

**Surface Sterilization of explants**

4 different strains of stevia rebaudiana (A, B, C, D) were taken from nursery; used as explant that was surface sterilized and cultured.

Leaves and nodal explants were washed with distilled water to remove dirt and debris, and then explants were washed with 2 % (v/v) detergent solution Teepol (Qualigen, India). Thereafter, it was surface sterilized with 0.5% (w/v) Bavistin (BASF, India Ltd.) and 0.1 % (w/v) Mercuric Chloride (Merck, India) followed by 4-5 washings with autoclaved distilled water in laminar air flow. Explants were cut into small segments by following protocol as mentioned by Sood et al 2009, 2010.

**Direct Organogenesis**

4 different strains of stevia rebaudiana (A, B, C, D); used as explants, that were surface sterilized and cultured. Small Incisions were given to the surface sterilized explants. These explants were cultured on MS media comprising of different growth hormone concentrations as stated above in Table2. The cultures were kept in the growth chambers for 20-25 days. The cultures were incubated at 25 ± 2 ºC, with 70 % relative humidity, 16 h day/8 h night photoperiod at photosynthetic photon flux density of 40 l mol m-2 s-1 provided by cool white fluorescent tubes (Philips, India) with sub culturing after every 4 weeks in a plant tissue culture chamber.

**Callus Induction and Regeneration**

4 different strains of stevia rebaudiana (A, B, C, D); used as explant, that were surface sterilized and cultured. Small Incisions were given to the surface sterilized explants. These explants were cultured on MS media comprising of different growth hormone concentrations as stated above in Table1. The cultures were kept in the growth chambers for 20-25 days. The cultures were incubated at 25 ± 2 °C, with 70 % relative humidity, 16 h day/8 h night photoperiod at photosynthetic photon flux density of 40 l mol m-2 s-1 provided by cool white fluorescent tubes (Philips, India) with sub culturing after every 4 weeks in a plant tissue culture chamber .Data would be collected for percentage of callus induction and number of days. The callus mass were cultured on shoot regeneration MS media comprising of different growth hormone concentrations as stated in Table 3. Proliferated multiple shoots were subculture on the same media to improve the number of shoots generation per shoot. Data would be gathered up for number of shoots formed per callus and days to form shoot and shoot length.

**Table 3: MS media supplemented with different concentrations of growth hormones for shoot regeneration from callus. (For four Different Strains *of S. rebaudiana*)**

| MS medium label | KN (mg/l) | IBA (mg/l) | IAA (mg/l) | BAP (mg/l) | NAA (mg/ml) |
|---|---|---|---|---|---|
| SR1 | 1 | 3 | | | |
| SR2 | 0.5 | 2.0 | | | |
| SR3 | 1 | | 3 | | |
| SR4 | 0.5 | | 2 | | |
| SR5 | 0.5 | | 1 | | |
| SR6 | 0.5 | | | 2 | |
| SR7 | 0.5 | | | 1 | |
| SR8 | | | | 1 | 0.5 |
| SR9 | | | | 1 | 2 |
| SR10 | | | | 0.5 | 1 |

.

**Different extraction of *Stevia rebaudiana:***

- **Plant Tissue Homogenization**

Fresh leaves are dried and grinded to fine particles. 50 ml of solvent is added to the flask and it is shaken vigorously for 5-10 minutes. Extract is filtered out and centrifugation is done for clarity of sample.

- **Serial exhaustive extraction**

A range of solvents from polar (methanol) to non-polar(hexane) is taken to ensure wide polarity range of compound extracted. Filtration is done after every 2 hours to with a change in the solvent.

- **Maceration**

Coarsely powdered plant is kept in contact with a solvent plugged into a flask. Frequent agitation is done until the soluble matter is dissolved completely.

**Sample preparation for Gas Chromatography –Mass Spectroscopy**

- **Plant material and extraction**

Two months old in vitro grown Stevia rebaudiana shoots labels as 2D sample and Greenhouse grown shoots were label as 4D sample. Each sample of *Stevia rebaudiana* shoots (100gm) were taken and dried

for 17 days under a temperature of 25 degree Celsius. After drying, the leaves were lyophilized using a lyophilized. Similarly with 1A and 1A-R for strain A extraction were prepared.

The extraction from shoots of Stevia rebaudiana were carried out by [42] done using various solvents. The most yield producing technique being ethyl acetate extraction. The preparation of ethyl acetate extract is done by heating the dried leaves of Stevia rebaudiana with ethyl acetate at boiling temperature. Ethyl acetate has biological characteristics which include low toxicity and medium polarity hence it the most preferred solvent. After heating the sample with the solvent, it is then filtered and freeze dried for later use. The samples are filtered using a Membrane filter (2µm**.)**

**Gas Chromatography –Mass spectroscopy**

The analyses were executed on a Shimadzu QP-2010 Plus with Thermal Desorption System TD 20 equipment with flame ionization detector and split/split less injector was at 290ºC and samples were injected using Auto sampler (1µl) with a split ratio 1:10. Capillary columns were used Rxi 5 ms (15m* 0.25 mm, with film thickness of 0.25µm) Restek. The temperature program was raised from 80ºC (1 min) up to 300ºC at the rate 5ºC/min, and the total run time was 49.98 minute. Helium was used a carrier gas at a flow rate of 5ºC/min. Detector temperature was set at 310ºC.To make the flame, hydrogen gas flow, 40ml/min, and air gas flow, 400mL/min and the carrier gas were He (45 ml/minute).In the analysis of samples were done by GC-2010 and GC program, the operating conditions were: column Oven temperature (50.0ºC), Injection Temperature (260ºC); Injection mode was split type Flow control mode was the linear velocity type (39.9cm/Sec); pressure (69kPa), total flow (16.3ml/min) and column flow (1.21ml/min) and run time (49.98 min); the carrier gas was helium at a constant flow of 1.1cc /minute. Ionization was performed by electrons at 70 EV: The scan range was 3333.start time was 4min and end time (49.98 minute); start m/z (40.00) and end m/z (650.00) with equilibrium time 0.5 minute. There was Ion Source temperature (230ºC), Interface Temp (270ºC), Solvent Cut Time (3.5 min). Detector Gain Mode was relative and threshold was 1000. GC solution software was used for data collection and calculation of all parameters.

**Quantification by High Performance Liquid Chromatography**

Fresh plantlets of *S. rebaudiana* were gently uprooted from jars and crushed in liquid nitrogen which produced a powdered sample of 100grams. This powdered sample was mixed with 10ml of 70% methanol as solvent. The samples were vortexed followed by sonication for 10 minutes. They were filtered using a 0.22 µm filter (Millipore). Analytical quantification of steviol glycosides from the extracts of powdered plant material. [44] Quantification was done on Waters HPLC system equipped with Waters 515 HPLC pumps, Autosampler, photodiode array detector and Empower software. Waters reverse phase C18 column (4.6mm x 250mm, 5µm) was used as the stationary phase and 10µl

of sample was injected into it with the mobile phase for analysis of secondary metabolite. The mobile phase was a mixture of MilliQ water (65%) and acetonitrile (35%), pH of 2.75 acidified by HCl. The linear gradient at a flow rate of 1 ml/min was equilibrated for 30 minutes at 210nm wavelength keeping the temperature at 25°C. The cycle time of analysis was 30 minutes. The solvents used for analysis were; Solvent A (ACN: water in the ratio of 65:35) and Solvent B (Methanol: water were in the ratio of 70:30).

## 9: RESULT:

- **Direct Organogenesis**

Best result for direct Organogenesis was seen in MS medium supplemented with growth hormones with a ratio of somehow different for different strain A,B,C,D as shown table [4,5,6,7] maximum shoot primordial were observed within 13 days in the media brought up above from nodal, internodes explants .After 6 days of incubation new shoots generation starts on most of the nodal explants was observed . The effect of different concentration of auxin and Cytokines combination on shoot formation, length of shoot, Number of shoots in stevia rebaudiana .For the Shoot generation or direct organogenesis nodes, internodes were cultured on MS medium supplemented with different concentration of different growth hormones. The nodes and internodes gives differential response to different PGRs combinations.

**Table 4: Direct Organogenesis of Strain A of S. rebaudiana on MS medium supplemented with different growth hormones.**

| Media Label | No of days to start shoot regeneration | Percent of shoot formation | No. of Shoot primordial |
|---|---|---|---|
| DO1 | 16 days ± 2 days | 82% | 4 shoots |
| DO2 | 17 days ± 2 days | 68% | 5 shoots |
| **DO3** | **13 days ± 2 days** | **72%** | **7 shoots** |
| DO4 | 15 days ± 2 days | 64% | 4 shoots |
| DO5 | 18 days ± 2 days | 54% | 5 shoots |
| DO6 | 20 days± 2 days | 62% | 6 shoots |

| | | | |
|---|---|---|---|
| DO7 | 19 days ± 2 days | 71% | 5 shoots |
| DO8 | 21 days ± 2 days | 76% | 4 shoots |
| DO9 | 18 days ± 2 days | 82% | 5 shoots |

*Values are the mean of three replicates each with 25 explants

*Percent of shoot formation (%) = No of shoot Produced / total no explants ×100

**Table 5: Direct Organogenesis of Strain B of S. rebaudiana on MS medium supplemented with different growth hormones.**

| Media Label | No of days to start shoot regeneration | Percent of shoot formation | No. of Shoot primordial |
|---|---|---|---|
| DO1 | 17± 2 days | 82% | 4 shoots |
| DO2 | 16± 2 days | 68% | 5 shoots |
| **DO3** | **14± 2 days** | **72%** | **7 shoots** |
| DO4 | 18± 2 days | 64% | 4 shoots |
| DO5 | 21± 2 days | 54% | 5 shoots |
| DO6 | 20± 2 days | 62% | 6 shoots |
| DO7 | 19± 2 days | 71% | 5 shoots |
| DO8 | 18± 2 days | 76% | 4 shoots |
| DO9 | 17± 2 days | 82% | 5 shoots |

*Values are the mean of three replicates each with 25 explants

*Percent of shoot formation (%) = No of shoot Produced / total no explants ×100

**Table 6: Direct Organogenesis of Strain C of S. rebaudiana on MS medium supplemented with different growth hormones.**

| Media Label | No of days to start shoot regeneration | Percent of shoot formation | No. of Shoot primordial |
|---|---|---|---|
| DO1 | 18± 2 days | 70% | 4 shoots |

| | | | |
|---|---|---|---|
| DO2 | 17± 2 days | 76% | 5 shoots |
| DO3 | 24± 2 days | 58% | 4 shoots |
| DO4 | 22± 2 days | 68% | 4 shoots |
| DO5 | 23± 2 days | 78% | 5 shoots |
| **DO6** | **17± 2 days** | **82%** | **6 shoots** |
| DO7 | 24± 2 days | 72% | 5 shoots |
| DO8 | 22± 2 days | 54% | 4 shoots |
| DO9 | 27± 2 days | 28% | 5 shoots |

*Values are the mean of three replicates each with 25 explants

*Percent of shoot formation (%) = No of shoot Produced / total no explants ×100

**Table 7: Direct Organogenesis of Strain D of S. rebaudiana on MS medium supplemented with different growth hormones.**

| Media Label | No of days to start shoot regeneration | Percent of shoot formation | No. of Shoot primordial |
|---|---|---|---|
| DO1 | 15± 2 days | 86% | 4 shoots |
| DO2 | 16± 2 days | 88% | 5 shoots |
| **DO3** | **17± 2 days** | **96%** | **8 shoots** |
| DO4 | 19± 2 days | 84% | 4 shoots |
| DO5 | 17± 2 days | 75% | 5 shoots |
| DO6 | 17± 2 days | 78% | 6 shoots |
| DO7 | 15± 2 days | 90% | 5 shoots |
| DO8 | 18± 2 days | 82% | 4 shoots |
| DO9 | 18± 2 days | 71% | 4 shoots |

*Values are the mean of three replicates each with 25 explants

*Percent of shoot formation (%) = No of shoot Produced / total no explants ×100

**Callus Induction**

Best results for callus induction was seen in MS medium supplemented with growth hormones with a ratio of IAA (3 mg/L) and Kinetin (1 mg/L) as shown in — Fig. 1B and Table 3 100% callusing was observed within 20 -25 days in the media brought up above from leaf explants. After 10 days of incubation, enlargement of most of the leaf explants was observed. The effect of different concentrations of auxin and cytokinin combination on percent callus formation, callus color, callus texture and callus growth in Stevia rebaudiana. For the induction of callus tissues, nodal and leaf segments were cultured on MS medium supplemented with different concentration of five PGR namely, IAA, NAA, 2, 4-dichlorophenoxyacetic acid (2, 4-D), BAP and KN, TDZ. The nodal and leaf segments gave a differential response to different PGR combinations. Morphogenetic differentiation of leaf segments started within 15–25 days after culture and made light green compact callus tissue. Here, maximum (100%) leaf explants produced callus in the media having 1.0 mg/l KN + 3.0 mg/l IAA. In case of leaf segment initiation of callus formation took place within 15 - 20 days of incubation. Calli were green compact from the leaf segment showed better response then in callus formation from internodal segments in different combinations.

**Table 8: Callus induction tissue from the nodal and leaf segments of *S. rebaudiana* on MS medium supplemented with different growth hormones. For strain A**

| Label | IBA (mg/l) | TDZ (mg/L) | KN (mg/L) | IAA (mg/l) | BAP (mg/ml) | 2,4, D (mg/ml) | NAA (mg/l) | Parameters for callus induction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Number of days for calli formation | Percent explants forming Calli (Initiation Freq) | Callus formation (%) | Callus color | Callus texture | Callus growth |
| CS1 | 3 | | 1 | | | | | 15 -20 days | 90% | 90% | Green | Compact | +++ |
| CS2 | 1 | | 3 | | | | | 18-20 days | 89% | 89% | Light Green | Compact | +++ |
| **CS3** | | | **1** | **3** | | | | **15-17 days** | **100%** | **100 %** | **Light Green** | **Compact** | +++++ |
| CS4 | | | | | 0.5 | | 2 | 18-20 days | 9% | 90% | Green | Granular | ++++ |
| CS5 | 0.5 | | | | | 2 | 0.5 | 20-22 days | 80% | 80% | Yellowish green | Granular | +++ |
| CS6 | 0.5 | 1 | | | | | | 18-20 days | 70% | 70% | Yellowish green | Granular | ++ |
| CS7 | 1 | 1.5 | | | | | | 15-17days | 80% | 80% | Yellowish green | Granular | +++ |
| CS8 | 1.5 | | 3 | | 1 | | | 18-20 days | 80% | 80% | Green | Compact | +++ |
| CS9 | 3 | | 1 | | | | | 15-17days | 90% | 90% | Green | Compact | ++++ |
| CS10 | | | | | 1 | | 2 | 20-22days | 60% | 60% | White | Granular | + |
| CS11 | 0.5 | | | | | | 2 | 18-20 days | 80% | 80% | Yellowish green | Granular | +++ |

*Values are the mean of three replicates each with 25 explants

*Initiation freq (%) = No of explants Produced / total no explants ×100

**Table 9: Callus induction tissue from the nodal and leaf segments of *S. rebaudiana* on MS medium supplemented with different growth hormones. For strain B**

| Label | IBA (mg/l) | TDZ (mg/L) | KN (mg/L) | IAA (mg/l) | BAP (mg/ml) | 2,4, D (mg/ml) | NAA (mg/l) | Parameters for callus induction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Number of days for calli formation | Percent explants forming Calli (Initiation Freq) | Callus formation (%) | Callus color | Callus texture | Callus growth |
| CS1 | 3 | | 1 | | | | | 20±2days | 74% | 74% | Green | Compact | +++ |
| CS2 | 1 | | 3 | | | | | 21± 2days | 89% | 89% | Light Green | Compact | +++ |
| CS3 | | | 1 | 3 | | | | 19± 2days | 86% | 86% | Light Green | Compact | +++++ |
| **CS4** | | | | | **0.5** | | **2** | **17 ± 2days** | **90%** | **90%** | **Green** | **Granular** | **++++** |
| CS5 | 0.5 | | | | | 2 | 0.5 | 22 ± 2days | 65% | 65% | Yellowish green | Granular | +++ |
| CS6 | 0.5 | 1 | | | | | | 19± 2days | 70% | 70% | Yellowish green | Granular | ++ |
| CS7 | 1 | 1.5 | | | | | | 17± 2days | 80% | 80% | Yellowish green | Granular | +++ |
| CS8 | 1.5 | | 3 | | 1 | | | 20 ±2days | 80% | 80% | Green | Compact | +++ |
| CS9 | 3 | | 1 | | | | | 19±2days | 90% | 90% | Green | Compact | ++++ |
| CS10 | | | | | 1 | | 2 | 20 ±2days | 60% | 60% | White | Granular | + |
| CS11 | 0.5 | | | | | | 2 | 20±2 days | 72% | 72% | Yellowish green | Granular | +++ |

*Values are the mean of three replicates each with 25 explants

*Initiation freq (%) = No of explants Produced / total no explants ×100

**Table 10: Callus induction tissue from the nodal and leaf segments of *S. rebudiana* on MS medium supplemented with different growth hormones. For strain C**

| Label | IBA (mg/l) | TDZ (mg/L) | KN (mg/L) | IAA (mg/l) | BAP (mg/ml) | 2,4, D (mg/ml) | NAA (mg/l) | Parameters for callus induction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Number of days for calli formation | Percent explants forming Calli (Initiation Freq) | Callus formation (%) | Callus color | Callus texture | Callus growth |
| CS1 | 3 | | 1 | | | | | 20±2 days | 86% | 86% | Green | Compact | +++ |
| CS2 | 1 | | 3 | | | | | 19± 2days | 70% | 68% | Light Green | Compact | +++ |
| **CS3** | | | **1** | **3** | | | | **17 ± 2days** | **92%** | **89%** | **Green** | **Compact** | **+++++** |
| CS4 | | | | | 0.5 | | 2 | 20±2 days | 90% | 90% | Green | Granular | ++++ |
| CS5 | 0.5 | | | | | 2 | 0.5 | 22±2 days | 80% | 76% | Yellowish green | Granular | +++ |
| CS6 | 0.5 | 1 | | | | | | 18±2days | 70% | 70% | White | Granular | ++ |
| CS7 | 1 | 1.5 | | | | | | 17±2days | 80% | 75% | Yellowish green | Granular | +++ |
| CS8 | 1.5 | | 3 | | 1 | | | 20 ±2days | 74% | 72% | Green | Compact | +++ |
| CS9 | 3 | | 1 | | | | | 17±2days | 90% | 82% | Green | Compact | ++++ |
| CS10 | | | | | 1 | | 2 | 20± 2days | 60% | 58% | White | Granular | + |
| CS11 | 0.5 | | | | | | 2 | 18±2days | 80% | 80% | Yellowish green | Granular | +++ |

*Values are the mean of three replicates each with 25 explants

*Initiation freq (%) = No of explants Produced / total no explants ×100

**Table11: Callus induction tissue from the nodal and leaf segments of *S. rebudiana* on MS medium supplemented with different growth hormones. For strain D**

| Label | IBA (mg/l) | TDZ (mg/L) | KN (mg/L) | IAA (mg/l) | BAP (mg/ml) | 2,4, D (mg/ml) | NAA (mg/l) | Parameters for callus induction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Number of days for calli formation | Percent explants forming Calli (Initiation Freq) | Callus formation (%) | Callus color | Callus texture | Callus growth |
| CS1 | 3 | | 1 | | | | | 15±2days | 90% | 90% | Green | Compact | +++ |
| CS2 | 1 | | 3 | | | | | 18±2days | 89% | 89% | Light Green | Compact | +++ |
| **CS3** | | | **1** | **3** | | | | **15±2days** | **100%** | **100 %** | **Light Green** | **Compact** | +++++ |
| CS4 | | | | | 0.5 | | 2 | 18±2days | 9% | 90% | Green | Granular | ++++ |
| CS5 | 0.5 | | | | | 2 | 0.5 | 20±2days | 80% | 78% | Yellowish green | Granular | +++ |
| CS6 | 0.5 | 1 | | | | | | 18±2 days | 70% | 70% | Yellowish green | Granular | ++ |
| CS7 | 1 | 1.5 | | | | | | 15±2days | 80% | 80% | Yellowish green | Granular | +++ |
| CS8 | 1.5 | | 3 | | 1 | | | 18±2days | 80% | 80% | Green | Compact | +++ |
| CS9 | 3 | | 1 | | | | | 15±2days | 90% | 90% | Green | Compact | ++++ |
| CS10 | | | | | 1 | | 2 | 20±2days | 60% | 60% | White | Granular | + |
| CS11 | 0.5 | | | | | | 2 | 18±2days | 80% | 80% | Yellowish green | Granular | +++ |

*Values are the mean of three replicates each with 25 explants

*Initiation freq (%) = No of explants Produced / total no explants ×100

**Shoot Regeneration**

Developed calli culture in regeneration media where the best results for shoot regeneration i.e. 5-6 shoot primordia was obtained on MS medium containing IBA (3 mg/L) and KN (1 mg/L) and BAP (1.5 mg/l) within 15-20 days as shown in ―Fig.1 and Table 4. Plant growth regulator in different combination induced shoots ranging from 2.4 – 5.1cm with a shoot length ranging from 3.25 – 11.30 cm. Shoot initiation were observed in the concentration of IBA 3 mg/L and Kinetin 1.0 mg/L. The maximum shoot formation was observed in medium containing IBA (3 mg/l) + BAP (1.5mg/L) + KN (1mg/l). Maximum number of shoots, buds and rapid elongation achieved on 1mg/ml BAP+ 1mg/ml IAA which is on the pattern of shoot formation achieved by Sweety and Mujumdar et al. (2016) in Stevia but it slow rate and less growth so optimized media for our study is IBA (3 mg/L) and KN (1 mg/L) and BAP (1.5mg/ml).

**Table 12: Shoot regeneration in *S. rebaudiana* on MS medium supplemented growth hormones for Strain A**

| Label | KN (mg/l) | IBA (mg/l) | IAA (mg/l) | BAP (mg/l) | NAA (mg/ml) | No. Of days for multiple shoot formation | Percent of Shoot formation | % of explant showing proliferation | % of calli forming multiple shoot formation |
|---|---|---|---|---|---|---|---|---|---|
| S1 | 1 | 3 | | | | 20 -25 days | 80% | 85% | 80% |
| S2 | 0.5 | 2 | | | | 19-23days | 70% | 73% | 70% |
| S3 | 1 | | 3 | | | 20 -25 days | 80% | 84% | 80% |
| S4 | 0.5 | | 2 | | | 18-22 days | 65% | 70% | 65% |
| S5 | 2 | | 1 | | | 20- 24 days | 70% | 75% | 70% |
| **S6** | **1** | **3** | | **1.5** | | **15-20days** | **90%** | **95%** | **90%** |
| S6 | 0.5 | 1 | | 2 | | 20-23 | 75% | 80% | 75% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | days | | | |
| S7 | 2 | | | 1 | | 18-20days | 85% | 80% | 85% |
| S8 | | | | 1 | 0.5 | 20-22 days | 80% | 84% | 80% |
| S9 | | | | 1 | 2 | 18 -21 days | 80% | 85% | 80% |
| S10 | | | | 0.5 | 1 | 20-25 days | 70% | 75% | 70% |

*Values are the mean of three replicates each with 25 explants.

**Table 13: Shoot regeneration in *S. rebaudiana* on MS medium supplemented growth hormones for Strain B**

| Label | KN (mg/l) | IBA (mg/l) | IAA (mg/l) | BAP (mg/l) | NAA (mg/ml) | No. Of days for multiple shoot formation | Percent of Shoot formation | % of explant showing proliferation | % of calli forming multiple shoot formation |
|---|---|---|---|---|---|---|---|---|---|
| S1 | 1 | 3 | | | | 21 -25 days | 80% | 85% | 80% |
| S2 | 0.5 | 2 | | | | 18-23days | 70% | 73% | 70% |
| S3 | 1 | | 3 | | | 20 -23 days | 80% | 84% | 80% |
| S4 | 0.5 | | 2 | | | 18-21 days | 65% | 70% | 65% |
| S5 | 2 | | 1 | | | 20- 22 days | 70% | 75% | 70% |
| **S6** | **1** | **3** | | **1.5** | | **15-18days** | **90%** | **85%** | **90%** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| S7 | 0.5 | 1 | | 2 | | 20-22days | 75% | 80% | 75% |
| S8 | 2 | | | 1 | | 18-22days | 85% | 80% | 85% |
| S9 | | | | 1 | 0.5 | 20-24 days | 80% | 84% | 80% |
| S10 | | | | 1 | 2 | 18 - 20days | 80% | 85% | 80% |
| S11 | | | | 0.5 | 1 | 20-25 days | 70% | 75% | 70% |

*Values are the mean of three replicates each with 25 explants

*percent of shoot formation= no of shoot formation/total no of explants on same medium ×100

*percentage of explant showing proliferation= no of shoot proliferated/total no of shoot formation×100

*percentage of calli forming multiple shoot formation= no of shoot generation from calli /total number of callus ×100

**Table 14: Shoot regeneration in *S. rebaudiana* on MS medium supplemented growth hormones for Strain C**

| Label | KN (mg/l) | IBA (mg/l) | IAA (mg/l) | BAP (mg/l) | NAA (mg/ml) | No. Of days for multiple shoot formation | Percent of Shoot formation | % of explant showing proliferation | % of calli forming multiple shoot formation |
|---|---|---|---|---|---|---|---|---|---|
| S1 | 1 | 3 | | | | 20 -25 days | 80% | 85% | 80% |
| S2 | 0.5 | 2 | | | | 19-23days | 70% | 73% | 70% |
| S3 | 1 | | 3 | | | 20 -25 days | 80% | 84% | 80% |
| S4 | 0.5 | | 2 | | | 18-22 | 65% | 70% | 65% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | days | | | |
| S5 | 2 | | 1 | | | 20- 24 days | 70% | 75% | 70% |
| **S6** | **1** | **3** | | **1.5** | | **15-20days** | **90%** | **95%** | **90%** |
| S7 | 0.5 | 1 | | 2 | | 20-23 days | 75% | 80% | 75% |
| S8 | 2 | | | 1 | | 18-20days | 85% | 80% | 85% |
| S9 | | | | 1 | 0.5 | 20-22 days | 80% | 84% | 80% |
| S10 | | | | 1 | 2 | 18 -21 days | 80% | 85% | 80% |
| S11 | | | | 0.5 | 1 | 20-25 days | 70% | 75% | 70% |

*Values are the mean of three replicates each with 25 explants

**Table 15: Shoot regeneration in *S. rebaudiana* on MS medium supplemented growth hormones for Strain D**

| Label | KN (mg/l) | IBA (mg/l) | IAA (mg/l) | BAP (mg/l) | NAA (mg/ml) | No. Of days for multiple shoot formation | Percent of Shoot formation | % of explant showing proliferation | % of calli forming multiple shoot formation |
|---|---|---|---|---|---|---|---|---|---|
| S1 | 1 | 3 | | | | 20 -22 days | 80% | 80% | 80% |
| S2 | 0.5 | 2 | | | | 19-21days | 70% | 73% | 70% |
| S3 | 1 | | 3 | | | 20 -25 days | 80% | 84% | 80% |
| S4 | 0.5 | | 2 | | | 18-22 days | 65% | 70% | 65% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| S5 | 2 | | 1 | | | 18- 24 days | 70% | 75% | 70% |
| **S6** | **1** | **3** | | **1.5** | | **15-20days** | **91%** | **91%** | **86%** |
| S7 | 0.5 | 1 | | 2 | | 21-23 days | 75% | 80% | 75% |
| S8 | 2 | | | 1 | | 22-25days | 85% | 80% | 85% |
| S9 | | | | 1 | 0.5 | 20-22 days | 82.6% | 84% | 80% |
| S10 | | | | 1 | 2 | 18 -23 days | 75.8% | 85% | 80% |
| S11 | | | | 0.5 | 1 | 20-24 days | 68% | 75% | 70% |

*Values are the mean of three replicates each with 25 explants

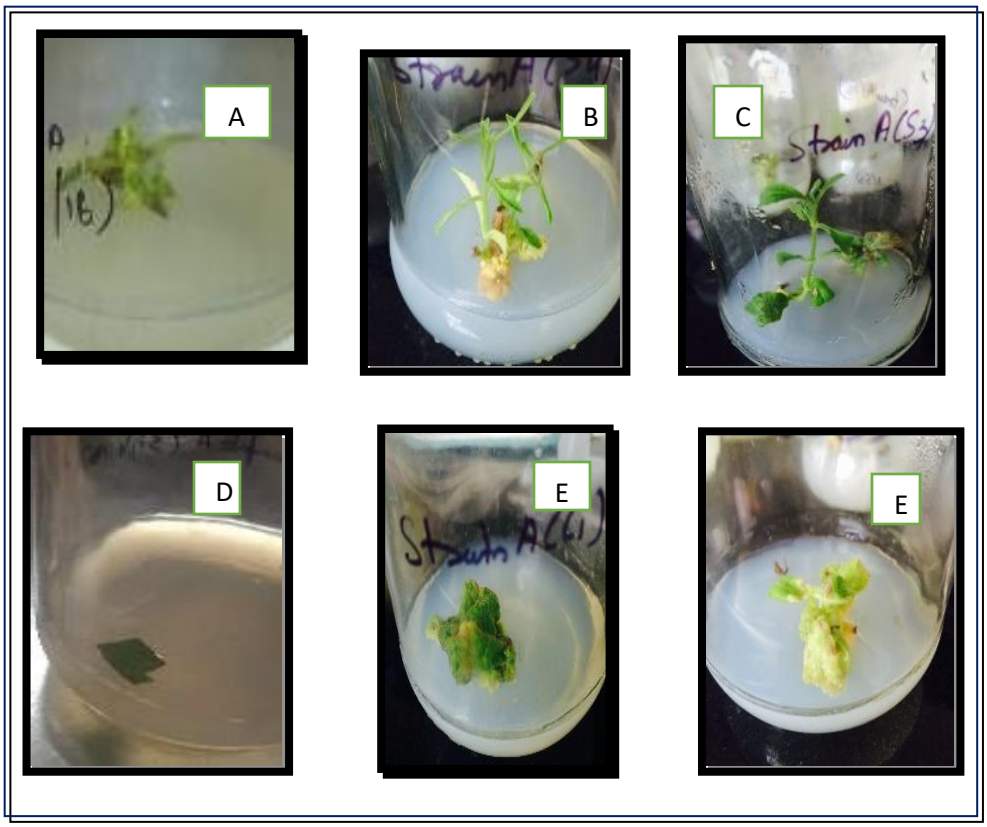


**Figure 4: *Stevia rebaudiana* strain A (A, B&C) direct organogenesis and (D, E & F) Indirect Organogenesis**.

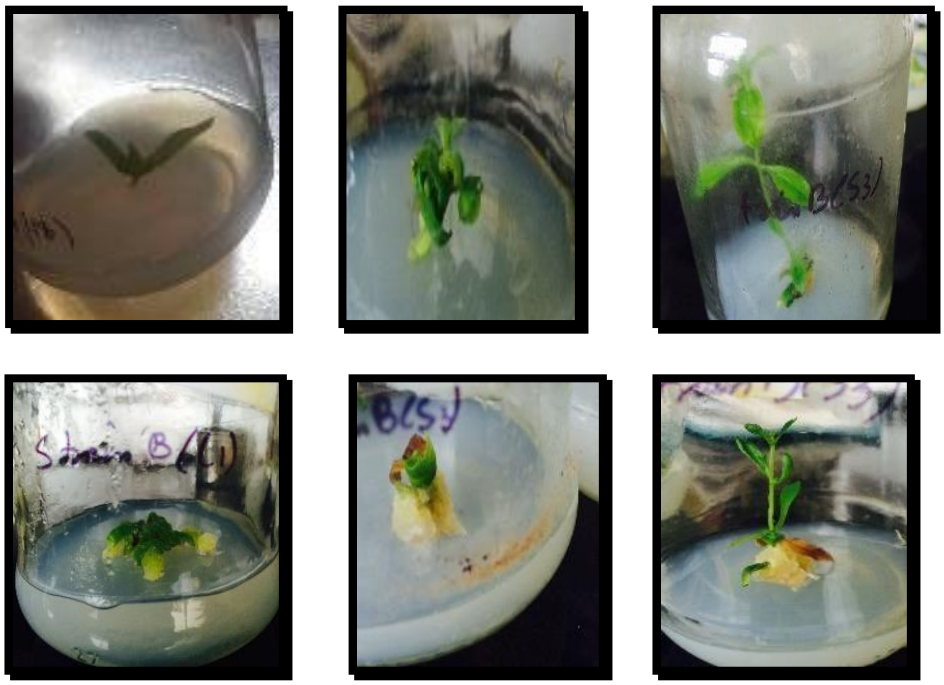

**Figure 5:** ***Stevia rebaudiana*** **strain B (A, B &C) direct organogenesis and (D,E & F) Indirect Organogenesis.**

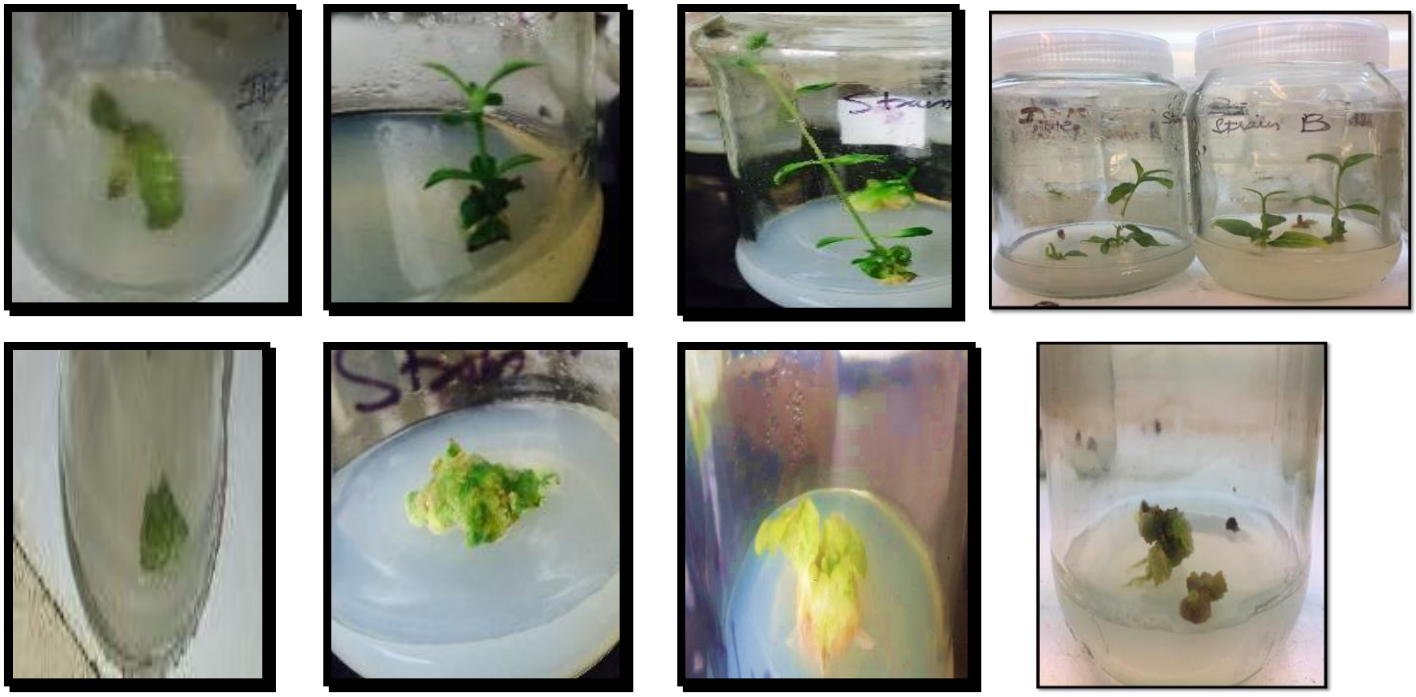

**Figure 6:** ***Stevia rebaudiana*** **strain C direct organogenesis and Indirect Organogenesis.**

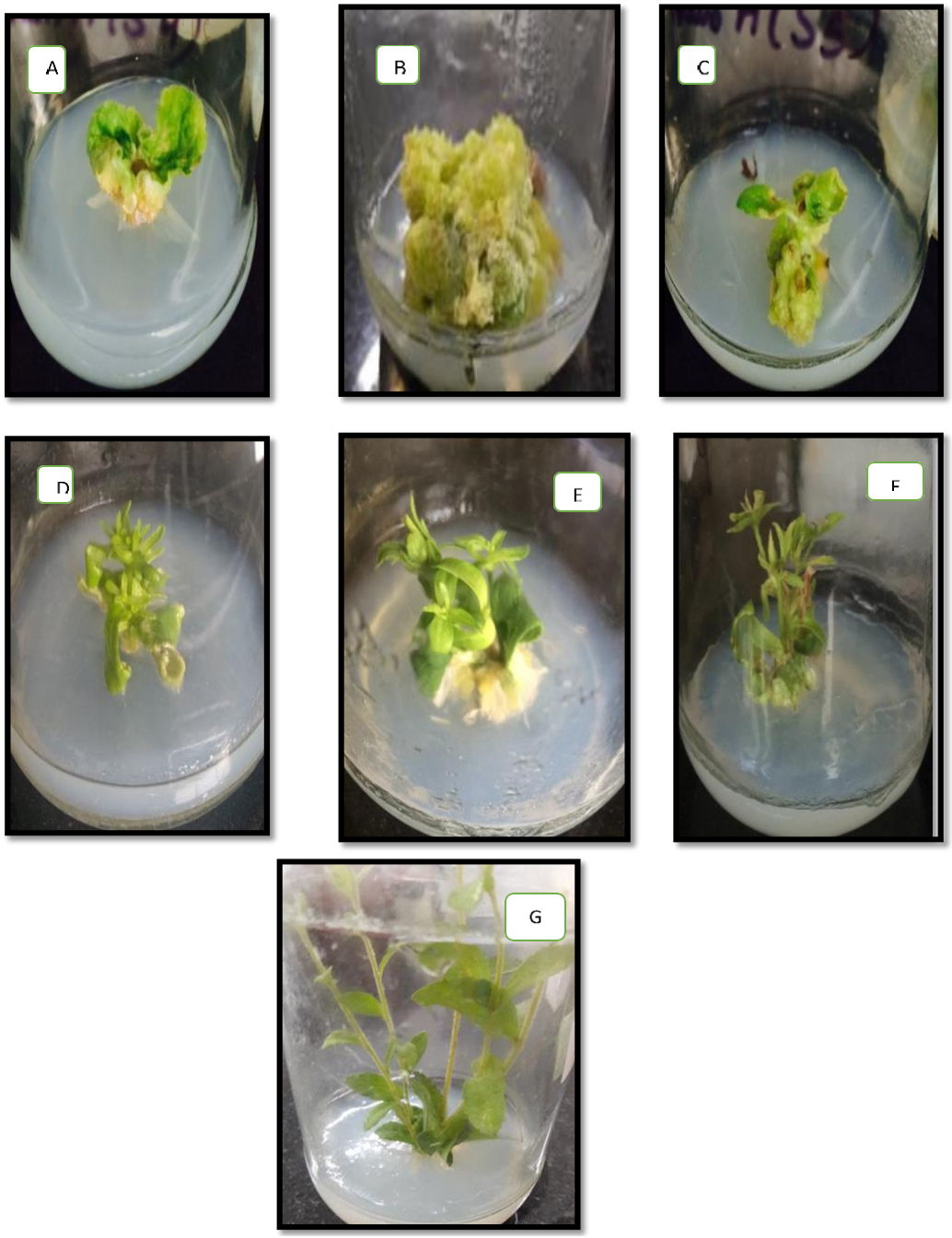


**Figure 7**: **Callus induction and shoot regeneration of *Stevia rebaudiana*** for strain D (A & B) Callus obtained on MS medium 3mg/l IAA+ 1 mg/ml KN within 17 days (C&D) Micro shoot formation in MS supplemented with 3mg/l kN+1mg/l BAP +1.5 mg/l IBA (E) Shoot growth after 15 days (F) 30 days old plantlet G) Two month old plantlets of *Stevia rebaudiana*.

**GCMS Result**

The GC-MS analysis has shown the presence of different phyto chemical compounds in the ethyl acetate extract of Stevia rebaudiana was carried out to observe that in-vitro grown young shoots of 2 months old culture are at par with the quality wise as compare to one year old shoot. A total of 86 compounds was identified, representing 100% of total ethyl acetate extract composition. From the results, it is evident that stevia rebaudiana contains various phyto component.

The GCMS Analysis of ethyl acetate extract shows the chromatographic profile of in vitro and in vivo culture of S. rebaudiana. As cultures show a complex mixture of compounds with fragmentation of longipinnate derivatives, labdenes, flavonoids, sterols, triterpenoids, monoterpenes like Geraniol, 2-berene, carvacrol. Sesquiterpenes were olopenone, T-Murolol, alpha-cubene and diterpene was neophyladiene, phytol, organic acids, monosaccharide and inorganic salts. Using ethyl extract n-alkene, n-alkanes, fatty acids, alcohols, terpenes were identified using GCMS.

Alpha-beta- selling, aroma dendrene, alpha-morphine, linden isomer are the starting material for the synthesis of fragrances and flavonoids. In one year old field grown shoots (4D) 86 molecules are identified(Table 6), whereas in two month old tissue cultured shoots (2D) 59 molecules were identified(Table 7) in which n-alkenes, n-alkanes, fatty acids, terpenes, diterpene, monoterpenes sesquiterpenes were identified. The results pertaining to GC-MS analysis of the ethyl acetate extract of *Stevia rebaudiana* leads to the identification of number of compounds. The compounds were identified through NSIT and Whiely library of mass spectrometry attached with GC. The GCMS spectrum confirmed the presence of various components with different retention times as shown in Fig 2 for 4D sample and in Fig 3 for 2D sample .The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compound's giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of compound which can be identified from the data library.

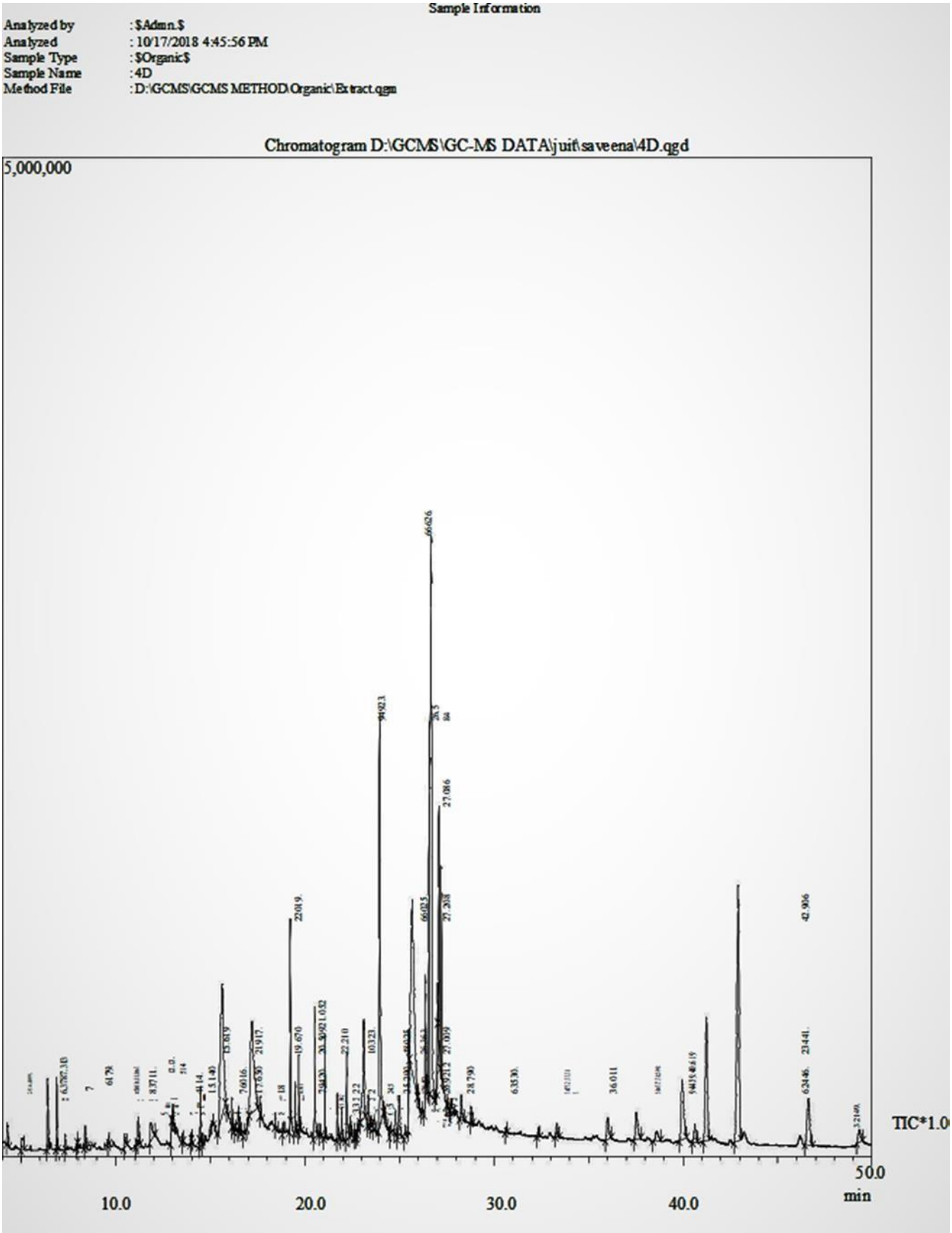


**Figure 8: GC MS chromatogram for field grown plant extract (4D sample) of *S. rebaudiana***

**Table 16: Bioactive compounds in field grown plant extract (4D sample) of *S. rebaudiana***

| Peak | Retention Time | Peak Area | Area % | Name of the compound | Molecular weight | Molecular formula | Compound nature |
|---|---|---|---|---|---|---|---|
| 1 | 4.253 | 479261 | 0.44 | 2-Propanone, 1,1-dimethoxy- ; Pyrolidine | 118 | C5H10O5 | Terpene |
| 2 | 5.109 | 466412 | 0.43 | PROPANE, 1,1-DIETHOXY-2-METHYL-; DIETHYL ACETAL | 146 | C8H18O2 | Organic coumpound |
| 3 | 6.378 | 1277391 | 1.17 | Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-; ALPHA-THUJENE | 136 | C10H16 | Alkene |
| 4 | 6.873 | 639086 | 0.59 | Butane, 1,1-diethoxy-3-methyl- | 160 | C9H20O2 | Organic coumpound |
| 5 | 7.313 | 166437 | 0.15 | BICYCLO[3.1.0]HEXANE, 4-METHYLENEs-1-(1-METHY;beta phellandren ;EINESE,SABINENE | 136 | C10H16 | Terpene |
| 6 | 7.930 | 36276 | 0.03 | dl-Threo-pentonic acid, 2,5-dideoxy-4-O-(phenylmethyl)-, m; Tphaniene ;BETA-PHELLANDREN | 238 | C13H18O4 | Cyclic monoterpene |
| 7 | 7.990 | 63005 | 0.06 | BICYCLO[4.1.0]HEPT-3-ENE, 3,7,7-TRIMETHYL-;3-CARENE | 136 | C10H16 | Monoterpene |
| 8 | 8.367 | 422438 | 0.39 | CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHENYL) ;BETA-LIMONENE | 136 | C10H16 | Monoterpene |
| 9 | 9.617 | 81917 | 0.08 | Alpha terpenolinene | 136 | C10H16 | TERPEN |
| 10 | 10.450 | 335799 | 0.31 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- | 144 | C6H8O4 | ALCHOL |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | methyl-;4-HPYRAN-4-OH | | | |
| 11 | 11.063 | 117606 | 0.11 | .beta.-D-Glucopyranoside, methyl 3,6-anhydro- | 176 | C7H12O5 | Carbohydrat E |
| 12 | 11.184 | 462635 | 0.43 | BENZOIC ACID, 2-HYDROXY-, METHYL ESTER,BETULA OIL | 152 | C8H8O3 | Ester |
| 13 | 11.837 | 699022 | 0.64 | 5-Hydroxymethylfurfural | 126 | C6H6O3 | Organic coumpound |
| 14 | 12.937 | 235448 | 0.22 | 2,3-Dimethyl-3-decanol | 186 | C12H2O6 | Primary alchol |
| 15 | 13.014 | 347236 | 0.32 | 1,2-BENZENEDICARBOXYLIC ACID, MONOBUTYL ES;MONOBUTYL PHTHALATE | 222 | C12H14O4 | Ester |
| 16 | 13.514 | 82697 | 0.08 | PHENOL, 2-METHOXY-4-(2-PROPENYL)- ;EUGENOL ACETATE | 164 | C10H12O2 | Flavinol |
| 17 | 13.972 | 71350 | 0.07 | DECANOIC ACID, ETHYL ESTER | 200 | C12H24O2 | Ester |
| 18 | 14.017 | 73030 | 0.07 | 2,4-DIISOPROPENYL-1-METHYL-1-VINYLCYCLOHEX;BETA ELEMEN | 204 | C15H24 | Alken |
| 19 | 14.417 | 29212 | 0.03 | 1-(2-METHYLENE-1PHENYLCYCLOPROPYL)ETHAN ; ERYTHRITOL TETRAACETATE | 172 | C12H12O | Organic oxidant |
| 20 | 14.476 | 483880 | 0.44 | BETA – CARYOPHYLLENE | 204 | C15H24 | Sequiterpen e |
| 21 | 14.578 | 80357 | 0.07 | 2-NORPINENE, 2,6- | 204 | C15H24 | Sequiterpen |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | DIMETHYL-6-(4-METHYL-3-PENTE;TRANS ALPHA BERGAMOTENE | | | E |
| 22 | 15.140 | 80313 | 0.07 | 4-(2,6,6-TRIMETHYL-1,3-CYCLOHEXADIEN-1-YL)-3-B;DEHYDRO-BETA-IONONE | 190 | C13H180 | SEQUITERPENE |
| 23 | 15.619 | 7577732 | 6.96 | D-Allose | 180 | C6H12O6 | Monosaccharides |
| 24 | 15.898 | 58631 | 0.05 | 9-OCTADECENOIC ACID (Z)-;9-OCTADECENOIC ACID | 282 | C18H34O2 | FATTY ACID |
| 25 | 16.150 | 272387 | 0.25 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-;NEROLIDOL | 222 | C15H26O | MONOTERPENE |
| 26 | 16.299 | 104264 | 0.10 | Isopropylphosphonic acid, fluoroanhydryde-, decyl ester | 266 | C13H28FO28 | Ester |
| 27 | 16.485 | 184666 | 0.17 | HEXADECANOIC ACID, ETHYL ESTER | 284 | C18H36 | Ester |
| 28 | 16.561 | 147959 | 0.14 | Caryophyllene oxide | 220 | C15H24O | SEQUITERPENE |
| 29 | 16.760 | 46478 | 0.04 | 3,9-Epoxy-p-mentha-1,8(10)-diene;ROSEFURAN,ALPHA-NAGINATENE | 150 | C10H14O | Terpene |
| 30 | 16.950 | 107866 | 0.10 | [1R-(1.ALPHA.,4.BETA.,5.BETA.)]-[5-(5-CHLORO-4,7,7-;3-METHYLNONAN-3-OL | 326 | C18H31ClOSi | ALCHOL |
| 31 | 17.219 | 593569 | 5.45 | 1,3,4,5- | 192 | C7H12O6 | FATTY |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 8 | | TETRAHYDROXY-CYCLOHEXANECARBOXYL;CHINIC ACID | | | ACID |
| 32 | 17.650 | 200231 | 0.18 | 2-Propenoic acid, tridecyl ester | 254 | C16H30 | FATTY ACID |
| 33 | 18.429 | 182551 | 0.17 | 01297107001 TETRANEURIN - A – DIOL;9-OCTADECENOIC ACID | 280 | C15H20O5 | TERPEN |
| 34 | 18.851 | 192946 | 0.18 | 2,3-Bis(1-methylallyl)pyrrolidine;Dehydrovomifolid | 179 | C12H21N | Terpene |
| 35 | 19.220 | 1586698 | 1.46 | Neophytadiene | 278 | C20H38 | DITERPENE |
| 36 | 19.476 | 476567 | 0.44 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol ;Phytol | 296 | C20H40O | DITERPENE |
| 37 | 19.670 | 574441 | 0.53 | Neophytadiene | 278 | C20H38 | DITERPENE |
| 38 | 19.743 | 114418 | 0.11 | Benzoic acid, 2-hydroxy-, phenylmethyl ester | 228 | C14H12O3 | Benzyl ester |
| 39 | 20.509 | 1465591 | 1.35 | n-Hexadecanoic acid | 256 | C16H32O2 | FATTY ACID |
| 40 | 20.794 | 101065 | 0.09 | HEPTADECANOIC ACID, ETHYL ESTER | 298 | C19H38O2 | Ester |
| 41 | 21.052 | 876348 | 0.81 | 1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10;sclareol | 290 | C19H34O | Diterpene alc0hol |
| 42 | 21.709 | 637554 | 0.59 | 1-Hexadecanol | 242 | C16H34O | TERPEN-ALCHOL |
| 43 | 21.942 | 429823 | 0.39 | 2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R | 296 | C20H | Primary alchol |
| 44 | 22.210 | 1325926 | 1.22 | cis,cis,cis-7,10,13-Hexadecatrienal | 234 | C16H26O | Unsaturated fatty |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | aldehyde |
| 45 | 22.413 | 237798 | 0.22 | Octadecanoic acid | 284 | C18H36O2 | FATTY ACID |
| 46 | 22.606 | 61019 | 0.06 | 1-Cyclohexene-1-acetaldehyde, 2,6,6-trimethyl- | 166 | C11H18O | Aldehyde |
| 47 | 22.733 | 88023 | 0.08 | 1,3-PROPANEDIOL, 2-METHYL-2-(1-METHYLPROPYL); cholоromethyl-2chlorododecanoate | 232 | C10H20N2O4 | Ester |
| 48 | 22.813 | 229554 | 0.21 | 8-AZABICYCLO[3.2.1]OCTANE-2-CARBOXYLIC ACID,;8-azabicycl | 289 | C16H19NO4 | Methyl ester |
| 49 | 23.103 | 2843075 | 2.61 | 1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4;globulol | 222 | C15H26O | Sesquiterpenids |
| 50 | 23.350 | 469621 | 0.43 | 1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4;duvatrendiol | 222 | C15H260 | KETONE |
| 51 | 23.470 | 93793 | 0.09 | 2-METHYL-4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-Y;13,15-octaosadiyne | 208 | C14H240 | ALKALYNE |
| 52 | 23.654 | 296799 | 0.27 | 1,4-Methanoazulen-7-ol, decahydro-1,5,5,8a-tetramethyl-, [1 | 222 | C15H26O | Terpene, Primary alcohol |
| 53 | 23.949 | 6983872 | 6.42 | 17.beta.-Hydroxy-6.alpha.-pentyl-4-nor-3,5-secoandrostan-3 | 378 | C24H42O3 | Acidmethyl ester |
| 54 | 24.217 | 262261 | 0.24 | 2,2,6-TRIMETHYL-1-(3-METHYL-BUTA-1,3- | 222 | C14H22O2 | Sesquiterpenoid enone |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | DIENYL)-7;aristolenepoxide | | | |
| 55 | 24.505 | 93043 | 0.09 | Thunbergol | 290 | C20H34O | Tetraprenyltoluquinols |
| 56 | 24.776 | 441759 | 0.41 | 1-Heptatriacotanol | 536 | C37H76O | Fatty alchol |
| 57 | 24.988 | 1193404 | 1.10 | Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-et | 250 | C16H26O2 | Ester |
| 58 | 25.290 | 232415 | 0.21 | Corymbolone | 236 | C15H24O2 | SesquiterpenE |
| 59 | 25.480 | 729807 | 0.67 | Thunbergol | 290 | C20H34O | Tetraprenyltoluquinols |
| 60 | 25.660 | 9538342 | 8.77 | 1,1,4A,7-TETRAMETHYL-2,3,4,4A,5,6,7,8-OCTAHYDRO;widdrol | 222 | C15H26O | Sesuiterpene |
| 61 | 25.991 | 245463 | 0.23 | 1-Naphthalenepropanol, .alpha.-ethenyldecahydro-2-hydroxy;Widdrol | 308 | C20H36O2 | Sesquiterpene |
| 62 | 26.183 | 44084 | 0.04 | DIOCTYL PHTHALATE | 390 | C24H38O4 | Diester |
| 63 | 26.363 | 1661004 | 1.53 | Thunbergol | 290 | C20H34O | Tetraprenyltoluquinols |
| 64 | 26.584 | 7125426 | 6.55 | 1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTA | 222 | C15H26O | Tannin |
| 65 | 26.666 | 14528897 | 13.35 | 8-propoxycedrane | 222 | C15H26O | Ether |
| 66 | 26.921 | 105434 | 0.10 | 1-PHENANTHRENEMETHANOL, 7-ETHENYL-1,2,3,4,4 | 274 | C19H30O | Primary alcohol |
| 67 | 27.009 | 800174 | 0.74 | Thunbergol | 290 | C20H34O | tetraprenyltoluquinols |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 68 | 27.086 | 2702939 | 2.48 | Thunbergol | 208 | C14H24O | tetraprenyltoluquinols |
| 69 | 27.208 | 3031808 | 2.79 | Hydroxydehydrostevic acid | 318 | C20H30O3 | Steviol |
| 70 | 27.522 | 109717 | 0.10 | Thunbergol | 290 | C20H34O | tetraprenyltoluquinols |
| 71 | 27.661 | 81447 | 0.07 | Eicosanoid | 282 | C20H42 | Fatty acids |
| 72 | 27.770 | 319240 | 0.29 | 3.alpha.,4.alpha.,9.beta.,11-Diepoxymuurolan-10-ol | 252 | C15H24O3 | Alchol |
| 73 | 28.269 | 593651 | 0.55 | Thunbergol | 290 | C20H34O | tetraprenyltoluquinols |
| 74 | 28.790 | 189433 | 0.17 | Squalene | 410 | C30H50 | Triterpene |
| 75 | 30.635 | 70728 | 0.06 | Myristyl myristate | 424 | C28H56O2 | Ester |
| 76 | 32.347 | 158363 | 0.15 | Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)- | 454 | C13H50O2 | Alkaloid |
| 77 | 33.313 | 326424 | 0.30 | dl-.alpha.-Tocopherol | 430 | C29H50O2 | Vitamin E |
| 78 | 36.011 | 812668 | 0.75 | Stigmasterol | 412 | C29H48O | Steroid derivative |
| 79 | 37.514 | 1130477 | 1.04 | STIGMAST-5-EN-3-OL, (3.BETA.)-;Beta- sitosterol | 414 | C29H50O | PRIMARY ALCHOL |
| 80 | 38.598 | 461389 | 0.42 | Globulol | 424 | C30H48O | Sesquiterpenoids |
| 81 | 39.944 | 2805398 | 2.58 | Lanosteryl acetate | 442 | C30H50O2 | Sterols |
| 82 | 40.619 | 649821 | 0.60 | Methyl commate A | 468 | C12H52O2 | Methyl coumpound |
| 83 | 41.234 | 4462326 | 4.10 | 4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B | 424 | C30H48O | Ethyl coumpound |
| 84 | 42.906 | 10378060 | 9.54 | Betulin | 442 | C30H50O2 | Triterpene |
| 85 | 46.624 | 228701 | 2.10 | Betulinaldehyde | 440 | C30H48O2 | Aldehyde |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | | | | | |
| 86 | 49.321 | 611805 | 0.56 | Isobutyric acid, tridecyl ester | 270 | C17H34O2 | Ester |
| | | 108818420 | 100.00 | | | | |

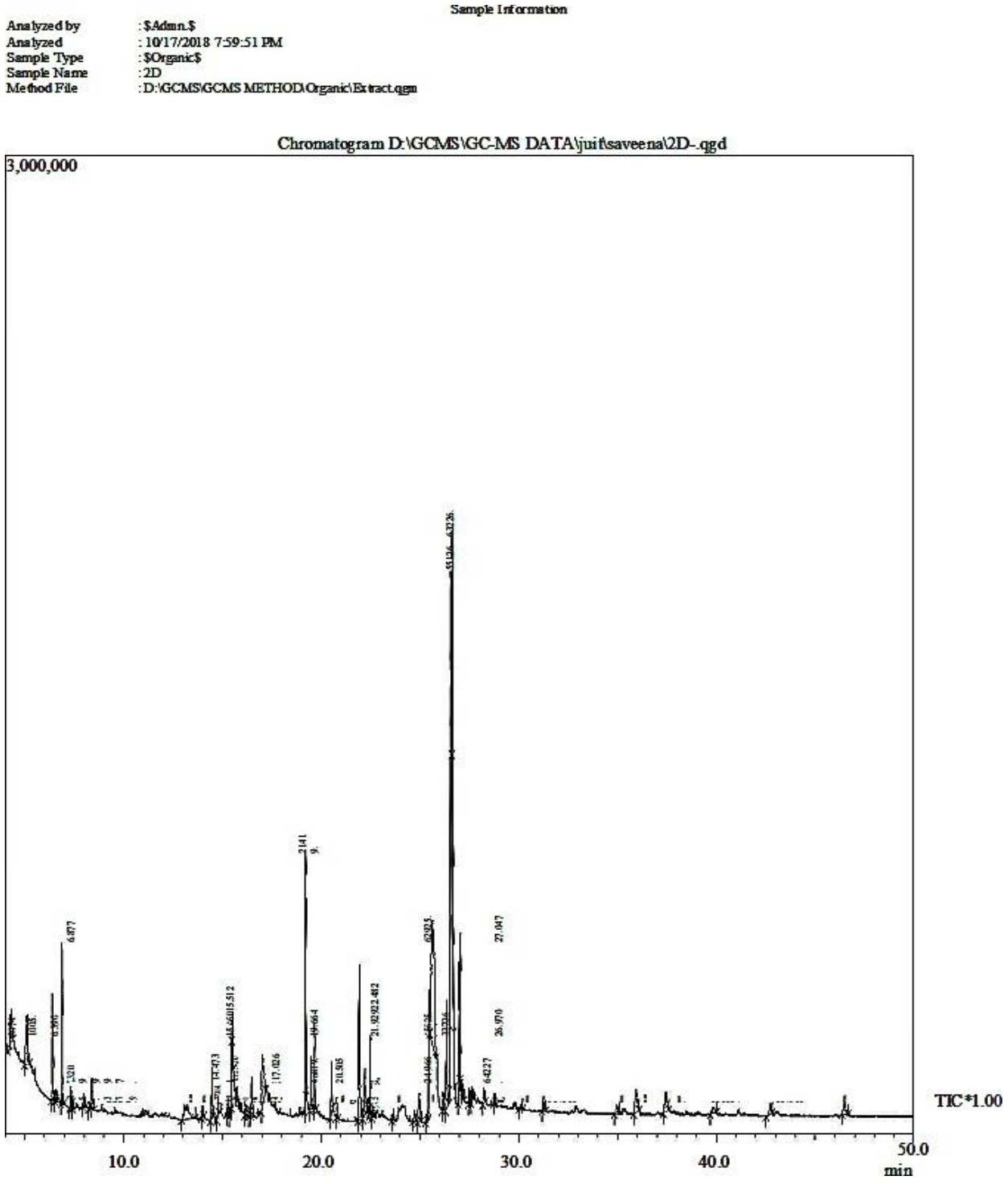


**Figure 9: GC MS chromatogram for tissue cultured plant extract (2D sample) of *S. rebaudiana***

**Table 17: Bioactive compounds in tissue cultured plant extract (2D sample) of *S. rebaudiana***

| Peak # | Retention Time | Area | Area % | Name | Chemical Formula | M.weight | Compound nature |
|---|---|---|---|---|---|---|---|
| 1 | 4.307 | 465187 | 1.56 | 2-Propanone, 1,1-dimethoxy- | C5H10O3 | 118 | Carboxylic acid |
| 2 | 5.100 | 795470 | 2.67 | Propane, 1,1-diethoxy-2-methyl- | C8H18O2 | 146 | Organic coumpound |
| 3 | 6.390 | 1120566 | 3.76 | Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-carenen | C10H16 | 136 | Monoterpene |
| 4 | 6.540 | 115245 | 0.39 | BICYCLO[3.1.1]HEPT-2-ENE, 2,6,6-TRIMETHYL- | C10H16 | 136 | Terpene |
| 5 | 6.877 | 941029 | 3.16 | Butane, 1,1-diethoxy-3-methyl- | C9H20O2 | 160 | Acetyl |
| 6 | 7.320 | 153054 | 0.51 | BICYCLO[3.1.0]HEXANE, 4-METHYLENE-1-(1-METHY | C10H16 | 136 | Terpene |
| 7 | 7.423 | 65002 | 0.22 | BICYCLO[3.1.1]HEPTANE, 6,6-DIMETHYL-2-METHYL | C10H16 | 136 | Terpene |
| 8 | 7.999 | 143342 | 0.48 | BICYCLO[4.1.0]HEPT-3-ENE, 3,7,7-TRIMETHYL- | C10H16 | 136 | Terpene |
| 9 | 8.267 | 60823 | 0.20 | BENZENE, 1-METHYL-3-(1-METHYLETHYL)- | C10H14 | 134 | Diterpene |
| 10 | 8.379 | 350670 | 1.18 | CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHENYL); Linonen | C10H16 | 136 | Monoterpene |
| 11 | 13.220 | 726168 | 2.44 | 1-Nitro-.beta.-d-arabinofuranose, tetraacetate | C13H17NO11 | 363 | Acetate |
| 12 | 13.987 | 105311 | 0.35 | UNDECANOIC ACID, ETHYL ESTER | C13H26O2 | 214 | Ester |
| 13 | 14.473 | 354168 | 1.19 | BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8- | C15H24 | 204 | Terpene |
| 14 | 14.773 | 144607 | 0.49 | (E)-.beta.-Famesene | C15H24 | 204 | Sesquiterpenes |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15 | 15.263 | 119815 | 0.40 | 1,6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-8- | C15H24 | 204 | Sesquiterpene |
| 16 | 15.380 | 38421 | 0.13 | 2-PENTENEDINITRILE, 2-[(1,1-DIMETHYLETHYL)AMI | C11H17N3 | 191 | |
| 17 | 15.460 | 238958 | 0.80 | Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1- | C15H24 | 204 | Phenylpropenes |
| 18 | 15.512 | 175726 | 0.59 | Phenol, 3,5-bis(1,1-dimethylethyl)- | C14H220 | 206 | Alkylphenols |
| 19 | 15.720 | 164083 | 0.55 | d-Gluco-heptulosan | C7H14O7 | 210 | Saccharide |
| 20 | 16.153 | 103683 | 0.35 | Nerolidol | C15H26O | 222 | Terpene |
| 21 | 16.340 | 64610 | 0.22 | 1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER | C13H15ClO4 | 270 | Ester |
| 22 | 16.488 | 349403 | 1.17 | DIETHYL PHTHALATE | C12H14O4 | 222 | Diterpene |
| 23 | 17.026 | 1122686 | 3.77 | 1,3,4,5-TETRAHYDROXY-CYCLOHEXANECARBOXYL | C7H12O6 | 192 | Carboxyl Acids |
| 24 | 19.214 | 1139959 | 3.83 | Neophytadiene | C20H40O | 296 | Terpene |
| 25 | 19.468 | 310960 | 1.04 | Neophytadiene | C20H40O | 296 | Terpene |
| 26 | 19.664 | 428373 | 1.44 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C22H42O2 | 338 | Diterpene alchol |
| 27 | 20.505 | 403671 | 1.35 | n-Hexadecanoic acid | C16H32O2 | 256 | Fatty acid |
| 28 | 20.788 | 124210 | 0.42 | HEPTADECANOIC ACID, ETHYL ESTER | C19H3802 | 298 | Ester |
| 29 | 21.929 | 1283739 | 4.31 | Phytol | C20H40O | 296 | Diterpen |
| 30 | 22.206 | 636187 | 2.13 | cis,cis,cis-7,10,13-Hexadecatrienal | C16H26O | 234 | Unsaturated fatty Acids |
| 31 | 22.365 | 43488 | 0.15 | (3E,7E,11E)-1-Isopropyl- | C20H34O | 290 | Diterpenoid |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | 4,8,12-trimethylcyclotetradeca-3,7, | | | |
| 32 | 22.427 | 51429 | 0.17 | 9,12-Octadecadienoic acid (Z,Z)- | C16H28O | 236 | Carboxylic acid |
| 33 | 22.482 | 356507 | 1.20 | (2E,6E)-9-(3,3-DIMETHYL-2-OXIRANYL)-3,7-DIMETHY | C21H30OS | 330 | Ester |
| 34 | 22.594 | 42347 | 0.14 | 1-Cyclohexene-1-acetaldehyde, 2,6,6-trimethyl- | C10H15BrO | 230 | Aldehyde |
| 35 | 23.634 | 48089 | 0.16 | NONANE, 3,7-DIMETHYL- | C13H28 | 184 | Hydrocarbons |
| 36 | 24.692 | 155836 | 0.52 | Heptadecane, 2,6,10,15-tetramethyl- | C29H60 | 408 | Methyl derivative |
| 37 | 24.966 | 450986 | 1.51 | 2,4,5,5,8A-PENTAMETHYL-4A,5,6,7,8,8A-HEXAHYDRO | C14H24O | 208 | Carboxylic Acid |
| 38 | 25.317 | 53692 | 0.18 | 7-Octylidenebicyclo[4.1.0]heptane | C12H18O | 178 | Alkane |
| 39 | 25.456 | 522118 | 1.75 | 1,1,4A,7-TETRAMETHYL-2,3,4,4A,5,6,7,8-OCTAHYDRO | C16H26O | 222 | Sesquiterpene |
| 40 | 25.629 | 4126866 | 13.85 | 1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTA | C16H26O2 | 250 | Volatile Coumpound |
| 41 | 26.165 | 127832 | 0.43 | 1,2-BENZENEDICARBOXYLIC ACID, DIISOOCTYL ESTer | C24H38O4 | 390 | Ester |
| 42 | 26.337 | 987880 | 3.32 | Thunbergol | C20H34O2 | 306 | Tetraprenyltoluquinols |
| 43 | 26.551 | 3460784 | 11.61 | 1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTA | C16H26O2 | 250 | Volatile coumpound |
| 44 | 26.632 | 2930526 | 9.83 | 1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTA | C15H26O | 222 | Volatile coumpound |
| 45 | 26.970 | 749170 | 2.51 | Thunbergol | C15H26O | 222 | Tetraprenyltoluquinols |
| 46 | 27.047 | 933371 | 3.13 | 4-(5,5-DIMETHYLSPIRO[2.5]OCT-4-YL)-2-BUTANONE | C22H36O2 | 332 | Ketone |
| 47 | 27.135 | 89572 | 0.30 | Thunbergol | C15H24O | 220 | Tetraprenyltoluquino |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | Ls |
| 48 | 27.494 | 85613 | 0.29 | Thunbergol | C15H26O | 290 | Tetraprenyltoluquinols |
| 49 | 27.642 | 83230 | 0.28 | 2-methyloctacosane | C24H49I | 464 | Acyclic Hydrocarbon |
| 50 | 28.221 | 172787 | 0.58 | 4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one | C15H26O | 222 | Ketone |
| 51 | 28.768 | 73178 | 0.25 | Squalene | C30H50 | 410 | Terpen |
| 52 | 30.141 | 50493 | 0.17 | EICOSANOIC ACID, METHYL ESTER | C21H42O2 | 326 | Ester |
| 53 | 31.246 | 154519 | 0.52 | Octacosyl acetate | C30H60O2 | 452 | Organic coumpound |
| 54 | 34.953 | 146561 | 0.49 | Octacosyl acetate | C30H60O2 | 452 | Organic coumpound |
| 55 | 35.925 | 529218 | 1.78 | Stigmasterol | C29H48O | 412 | Sterol |
| 56 | 37.443 | 485582 | 1.63 | .gamma.-Sitosterol | C29H50O | 414 | Terpene |
| 57 | 39.863 | 167659 | 0.56 | Thunbergol | C20H34O | 290 | Tetraprenyltoluquinols |
| 58 | 42.737 | 224389 | 0.75 | 1,4-Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol | C15H26O | 222 | Primary alchol |
| 59 | 46.471 | 249533 | 0.84 | LUP-20(29)-ENE-3,28-DIOL, (3.BETA.)- | C30H50O2 | 442 | Alchol |
| | | 29798381 | 100.00 | | | | |

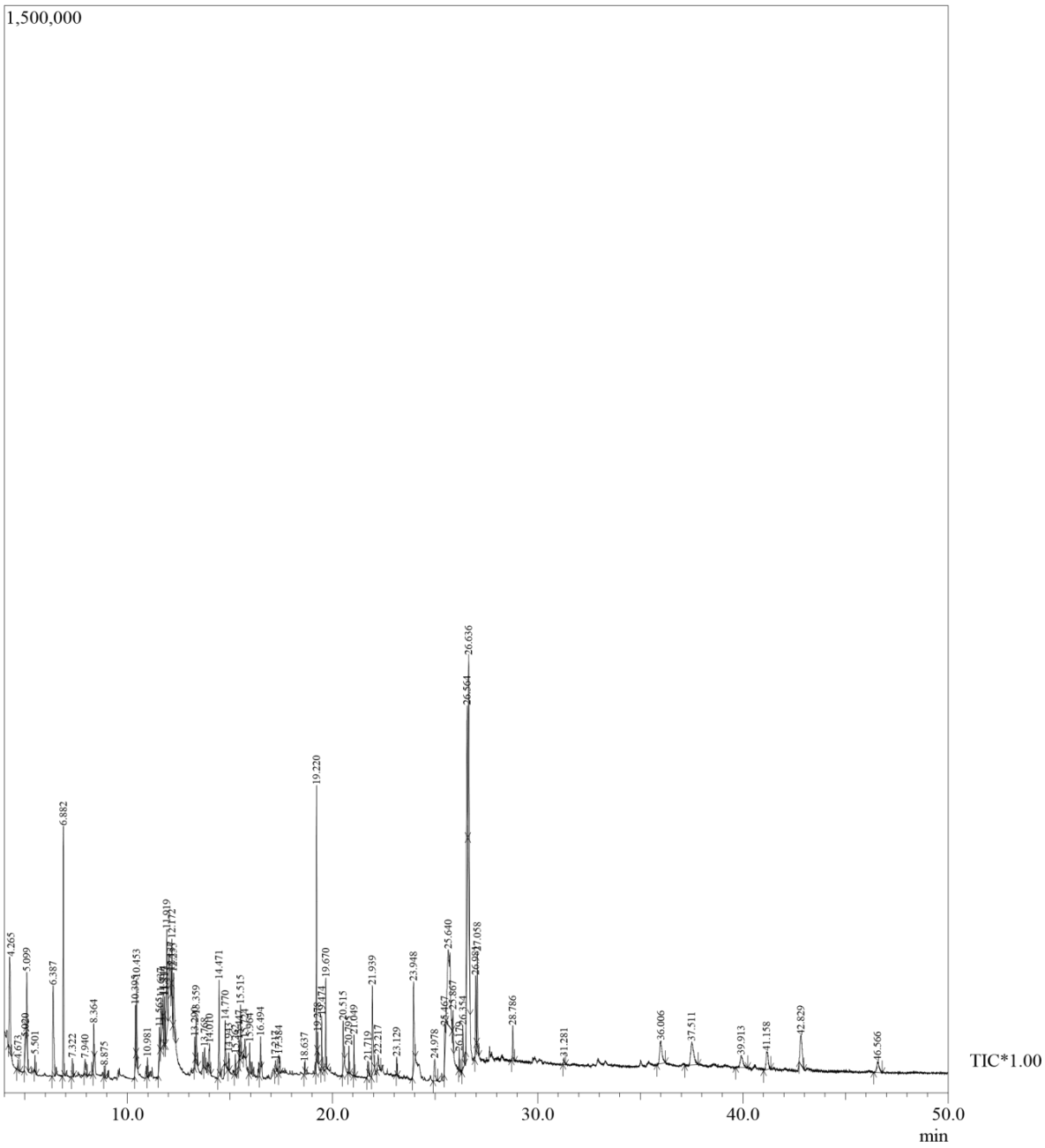


**Figure 10: GC MS chromatogram for tissue cultured plant extract (1A sample) of *S. rebaudiana***

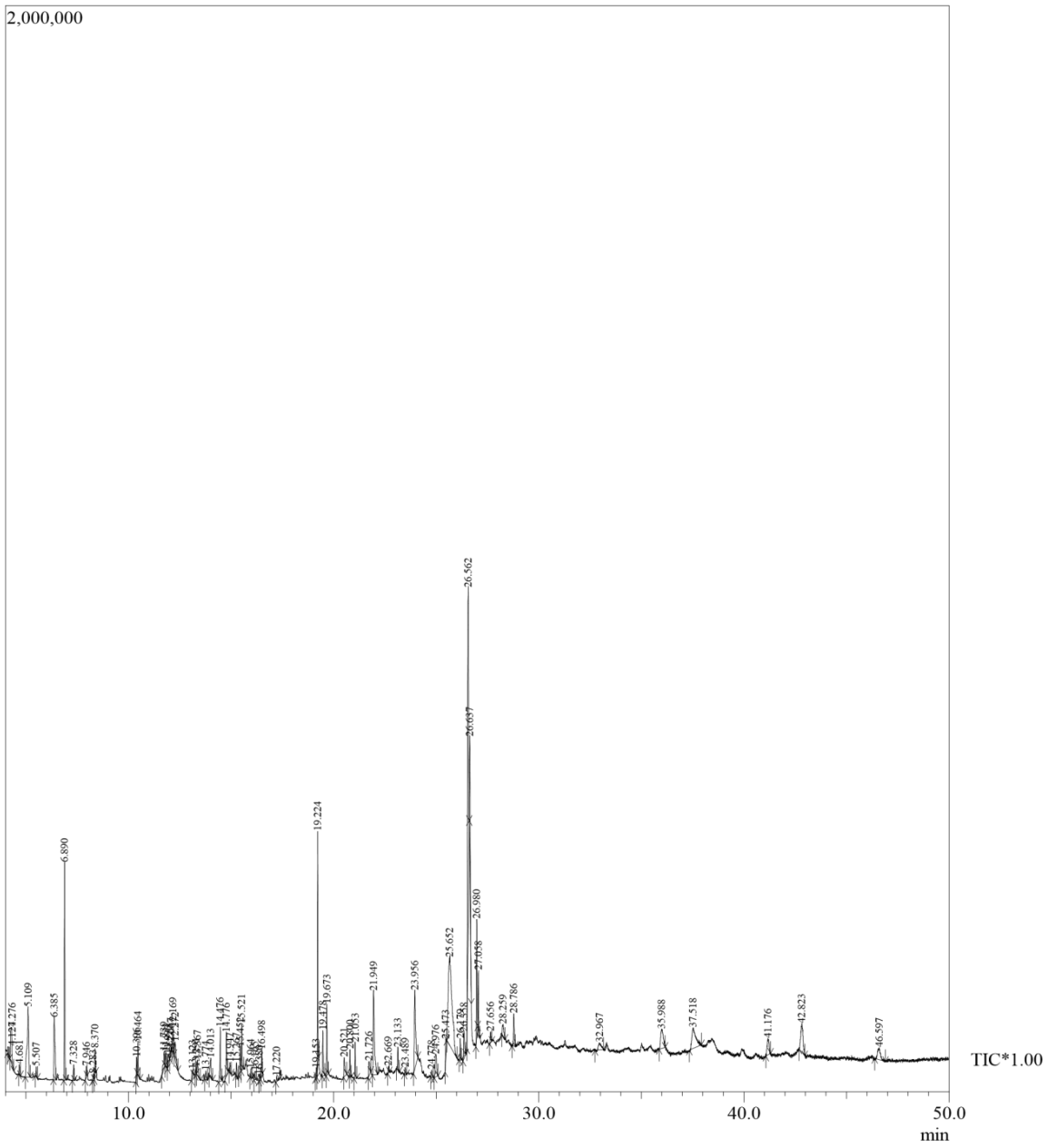


**Figure 11: GC MS chromatogram for tissue cultured plant extract (1A sample) of *S. rebaudiana***

**Table 18 : Bioactive compounds in tissue cultured plant extract (2D sample) of *S. rebaudiana***

| Peak | Retention Time | Area | Area % | Name | Chemical Formula | Molecular Weight | Compound Nature |
|---|---|---|---|---|---|---|---|
| 1 | 4.265 | 470441 | 3.51 | 2-Propanone, 1,1-dimethoxy- | C5H10O3 | 118 | Fatty Acid |
| 2 | 4.673 | 27763 | 0.21 | ACETIC ACID, 1-METHYLETHYL ESTER | C5H10O2 | 102 | Ester |
| 3 | 5.020 | 66317 | 0.50 | ETHANE, 1,1,1-TRINITRO- | C2H3N3O6 | 165 | Ether |
| 4 | 5.099 | 222269 | 1.66 | Propane, 1,1-diethoxy-2-methyl- | C8H18O2 | 146 | Ether |
| 5 | 5.501 | 28570 | 0.21 | 1-BUTANOL, 3-METHYL-, ACETATE | C7H14O2 | 130 | Ester |
| 6 | 6.387 | 472130 | 3.52 | 5-ISOPROPYL-2-METHYLBICYCLO[3.1.0]HEX-2-ENE | C10H16 | 136 | Monoterpene |
| 7 | 6.882 | 698020 | 5.21 | Butane, 1,1-diethoxy-3-methyl- | C9H20O2 | 160 | Ether |
| 8 | 7.322 | 51746 | 0.39 | BICYCLO[3.1.0]HEXANE, 4-METHYLENE-1-(1-METHY | C10H16 | 136 | Monoterpene |
| 9 | 7.940 | 90096 | 0.67 | Butane, 1-chloro-4-(1-ethoxyethoxy)- | C8H17ClO2 | 180 | Ether |
| 10 | 8.364 | 112953 | 0.84 | CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHENYL) | C10H16 | 136 | Monoterpene |
| 11 | 8.875 | 30194 | 0.23 | BICYCLO[3.1.0]HEX-2-ENE, 2-METHYL-5-(1-METHYL | C10H16 | 136 | Monoterpene |
| 12 | 10.395 | 141868 | 1.06 | 6-Octenal, 3,7-dimethyl-, (R)- | C10H18O | 154 | Ketone |
| 13 | 10.453 | 187697 | 1.40 | 6-Octenal, 3,7-dimethyl-, (R)- | C10H18O | 154 | Ketone |
| 14 | 10.981 | 40328 | 0.30 | 3-CYCLOHEXEN-1-OL, 4-METHYL-1-(1-METHYLETHY | C10H18O | 154 | Ketone |
| 15 | 11.565 | 129497 | 0.97 | Citronellol | C10H20O | 156 | Monoterpene |
| 16 | 11.637 | 172232 | 1.29 | 3,7-DIMETHYL-7-OCTEN-1-OL # | C10H20O | 156 | Monoterpene |
| 17 | 11.744 | 42747 | 0.32 | 2,6-Octadienal, 3,7-dimethyl-, (Z)- | C10H16O | 152 | Monoterpene |
| 18 | 11.817 | 121576 | 0.91 | 2,6-Octadienal, 3,7-dimethyl-, (Z)- | C10H16O | 152 | Monoterpene |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 19 | 11.919 | 415724 | 3.10 | 2,6-OCTADIEN-1-OL, 3,7-DIMETHYL-, (E)- | C10H18O | 154 | Monoterpene |
| 20 | 12.137 | 24612 | 0.18 | 4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)- | C10H18O | 154 | Monoterpene |
| 21 | 12.172 | 90363 | 0.67 | 2,6-Octadienal, 3,7-dimethyl-, (E)- | C10H16O | 152 | Monoterpene |
| 22 | 12.255 | 252675 | 1.89 | Citral | C10H16O | 152 | Monoterpene |
| 23 | 13.290 | 54926 | 0.41 | 6-Octen-1-ol, 3,7-dimethyl-, acetate | C12H22O2 | 198 | Carboxylic Ester |
| 24 | 13.359 | 147601 | 1.10 | 6-Octen-1-ol, 3,7-dimethyl-, acetate | C12H22O2 | 198 | Carboxylic Ester |
| 25 | 13.768 | 74451 | 0.56 | 2,6-OCTADIEN-1-OL, 3,7-DIMETHYL-, ACETATE | C12H20O2 | 196 | Carboxylic Ester |
| 26 | 14.010 | 97587 | 0.73 | Ethyl cyclohexanepropionate | C11H20O2 | 184 | Ester |
| 27 | 14.471 | 282436 | 2.11 | BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8- | C15H24 | 204 | Sesquiterpene |
| 28 | 14.770 | 125206 | 0.93 | (E)-.beta.-Famesene | C15H24 | 204 | Sesquiterpene |
| 29 | 14.943 | 41691 | 0.31 | Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene- | C10H16 | 136 | Monoterpene |
| 30 | 15.262 | 59181 | 0.44 | 1,6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-8- | C15H24 | 204 | Sesquiterpene |
| 31 | 15.447 | 47645 | 0.36 | GERMACRENE B | C15H24 | 204 | Sesquiterpene |
| 32 | 15.515 | 137207 | 1.02 | Phenol, 3,5-bis(1,1-dimethylethyl)- | C14H22O | 206 | Aromatic |
| 33 | 15.724 | 71052 | 0.53 | 4-[(2,3,5,6-TETRAMETHYLPHENYL)SULFONYL]-2-BU | C15H23N3O2S2 | 341 | Aromatic Ether |
| 34 | 15.964 | 89419 | 0.67 | BENZENE, 1,2,3-TRIMETHOXY-5-(2-PROPENYL)- | C12H16O3 | 208 | Aromatic Ether |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 35 | 16.494 | 105305 | 0.79 | DIETHYL PHTHALATE | C12H14O4 | 222 | Aromatic |
| 36 | 17.217 | 41000 | 0.31 | 2-Heptene, 5-ethyl-2,4-dimethyl- | C11H22 | 154 | Diterpene |
| 37 | 17.384 | 82959 | 0.62 | 1-NAPHTHALENOL, 1,2,3,4,4A,7,8,8A-OCTAHYDRO-1, | C15H26O | 222 | Lipid |
| 38 | 18.637 | 17308 | 0.13 | 2,3-DEHYDRO-.ALPHA.-ISOMETHYLIONONE | C14H20O | 204 | Ester |
| 39 | 19.220 | 577529 | 4.31 | Neophytadiene | C20H38 | 278 | Diterpene |
| 40 | 19.278 | 40932 | 0.31 | (2E)-3,7,11,15-TETRAMETHYL-2-HEXADECENE # | C20H40 | 280 | Diterpene |
| 41 | 19.474 | 132062 | 0.99 | Neophytadiene | C20H38 | 278 | Diterpene |
| 42 | 19.670 | 213247 | 1.59 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C20H40O | 296 | Diterpene |
| 43 | 20.515 | 179520 | 1.34 | HEXADECANOIC ACID | C16H32O2 | 256 | Carboxylic Acid |
| 44 | 20.795 | 67787 | 0.51 | ETHYL PENTADECANOATE | C17H34O2 | 270 | Ester |
| 45 | 21.049 | 84061 | 0.63 | 1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10 | C20H34O | 290 | Ether |
| 46 | 21.719 | 56915 | 0.42 | 1-UNDECANOL | C11H24O | 172 | Alkane |
| 47 | 21.939 | 397655 | 2.97 | Phytol | C20H40O | 296 | Alkane |
| 48 | 22.217 | 82946 | 0.62 | Cyclooctane-1,4-diol, cis | C8H16O2 | 144 | Alkane |
| 49 | 23.129 | 40514 | 0.30 | LABDA-8(17),13E-DIEN-15-OIC ACID | C20H32O2 | 304 | Diterpene |
| 50 | 23.948 | 402450 | 3.00 | Androstane-3,17-diol, 17-methyl-, (3.beta.,5.alpha.,17.beta.)- | C20H34O2 | 306 | Alkane |
| 51 | 24.978 | 133432 | 1.00 | 2,6,10,14-HEXADECATETRAEN-1-OL, 2,6,10,14-TETRA | C26H38OS | 398 | Isoprenoid |
| 52 | 25.467 | 106622 | 0.80 | 13-TETRADECEN-2-YN-1-OL | C14H24O | 208 | Aromatic |
| 53 | 25.640 | 1075024 | 8.02 | 1,1,4A,7-TETRAMETHYL- | C15H26O | 222 | Sesquiterpene |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | 2,3,4,4A,5,6,7,8-OCTAHYDRO | | | |
| 54 | 25.867 | 86635 | 0.65 | 2-PHENANTHRENECARBOXALDEHYDE, 1,2,3,4,4A,4B | C19H30O3 | 306 | Diterpene |
| 55 | 26.179 | 55586 | 0.41 | 1,2-BENZENEDICARBOXYLIC ACID | C24H38O4 | 390 | Carboxylic Acid |
| 56 | 26.354 | 200912 | 1.50 | 1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4 | C15H26O | 222 | Aromatic |
| 57 | 26.564 | 1098847 | 8.20 | 1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTA | C15H26O | 222 | Aromatic |
| 58 | 26.636 | 1096307 | 8.18 | 1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTA | C15H26O | 222 | Aromatic |
| 59 | 26.981 | 208900 | 1.56 | Thunbergol | C20H34O | 290 | Diterpenoid |
| 60 | 27.058 | 247793 | 1.85 | Thunbergol | C20H34O | 290 | Diterpenoid |
| 61 | 28.786 | 121620 | 0.91 | Squalene | C30H50 | 410 | Terpene |
| 62 | 31.281 | 28062 | 0.21 | Cycloundecane, 1,1,2-trimethyl- | C14H28 | 196 | Acyclic Olefins |
| 63 | 36.006 | 255345 | 1.91 | 1-Heptatriacotanol | C37H76O | 536 | Fatty Alcohol |
| 64 | 37.511 | 311971 | 2.33 | 1-Heptatriacotanol | C37H76O | 536 | Fatty Alcohol |
| 65 | 39.913 | 148083 | 1.11 | Methanol, [6,8,9-trimethyl-4-(1-propenyl)-3-oxabicyclo[3.3. | C15H24O2 | 236 | Sesquiterpene |
| 66 | 41.158 | 179702 | 1.34 | 24-Noroleana-3,12-diene | C29H46 | 394 | Aromatic |
| 67 | 42.829 | 286744 | 2.14 | 1,4-Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol | C15H26O | 222 | Sesquiterpene |
| 68 | 46.566 | 115031 | 0.86 | 1,2-Pentanediol, 5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a- | C20H35BrO3 | 402 | Isoprene |
| | | 13397026 | 100.00 | | | | |

**Table 19 : Bioactive compounds in tissue cultured plant extract (2D sample) of *S. rebaudiana***

| Peak | Retention Time | Area | Area % | Name | Chemical Formula | Molecular Weight | Nature |
|---|---|---|---|---|---|---|---|
| 1 | 4.127 | 36992 | 0.24 | Butanoic acid, heptyl ester | C11H2202 | 186 | Ester |
| 2 | 4.276 | 295543 | 1.89 | 2-Propanone, 1,1-dimethoxy | C5H10O3 | 118 | Ketone |
| 3 | 4.681 | 44902 | 0.29 | ACETIC ACID, 1-METHYLETHYL ESTER | C5H10O2 | 102 | Ester |
| 4 | 5.109 | 674478 | 4.31 | Propane, 1,1-diethoxy-2-methyl- | C8H18O2 | 146 | Ether |
| 5 | 5.507 | 34016 | 0.22 | 1-BUTANOL, 3-METHYL-, ACETATE | C7H14O2 | 130 | Ester |
| 6 | 6.385 | 435234 | 2.78 | 5-ISOPROPYL-2-METHYLBICYCLO[3.1.0]HEX-2-ENE | C10H16 | 136 | Monoterpene |
| 7 | 6.890 | 731341 | 4.67 | Butane, 1,1-diethoxy-3-methyl | C9H20O2 | 160 | Ether |
| 8 | 7.328 | 55234 | 0.35 | CYCLOHEXENE, 3-METHYLENE-6-(1-METHYLETHYL | C10H16 | 136 | Monoterpene |
| 9 | 7.946 | 49489 | 0.32 | Butane, 1-chloro-4-(1-ethoxyethoxy | C8H17ClO2 | 180 | Ether |
| 10 | 8.283 | 28362 | 0.18 | BICYCLO[4.1.0]HEPT-3-ENE-2-THIOL, 3,7,7-TRIMETHY | C10H16S | 168 | Monoterpene |
| 11 | 8.370 | 101158 | 0.65 | CYCLOHEXENE, 1-METHYL-4- | C10H16 | 136 | Monoterpene |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | (1-METHYLETHENYL | | | |
| 12 | 10.396 | 68993 | 0.44 | 6-Octenal, 3,7-dimethyl-, (R)- | C10H18O | 154 | Monoterpene |
| 13 | 10.464 | 130233 | 0.83 | 6-Octenal, 3,7-dimethyl-, (R)- | C10H18O | 154 | Monoterpene |
| 14 | 11.739 | 133930 | 0.86 | Citral | C10H16O | 152 | Monoterpene |
| 15 | 11.831 | 79823 | 0.51 | Citral | C10H16O | 152 | Monoterpene |
| 16 | 11.933 | 77524 | 0.50 | Citronellol | C10H20O | 156 | Monoterpene |
| 17 | 12.067 | 45495 | 0.29 | (3,5-DIMETHYL-1H-PYRAZOL-1-YL)(2,2-DIOXIDO-2LA | C6H9N5O2 | 183 | Aromatic |
| 18 | 12.169 | 102705 | 0.66 | Citral | C10H16O | 152 | Monoterpene |
| 19 | 12.272 | 154032 | 0.98 | 2,6-Octadienal, 3,7-dimethyl-, (E)- | C10H16O | 152 | Monoterpene |
| 20 | 13.123 | 41056 | 0.26 | 1,2,3-Propanetriol, 1-acetate | C5H10O4 | 134 | Ester |
| 21 | 13.291 | 22625 | 0.14 | 6-OCTEN-1-OL, 3,7-DIMETHYL-, ACETATE | C12H22O2 | 198 | Ester |
| 22 | 13.367 | 89063 | 0.57 | 6-OCTEN-1-OL, 3,7-DIMETHYL-, | C12H22O2 | 198 | Ester |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | ACETATE | | | |
| 23 | 13.774 | 55622 | 0.36 | 2,6-OCTADIEN-1-OL, 3,7-DIMETHYL-, ACETATE | C12H20O2 | 196 | Aromatic |
| 24 | 14.013 | 139378 | 0.89 | (Z)- Ethyl cyclohexanepropionate | C11H20O2 | 184 | Ester |
| 25 | 14.476 | 219630 | 1.40 | BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8- | C15H24 | 204 | Sesquiterpene |
| 26 | 14.947 | 32340 | 0.21 | 1,3,6-OCTATRIENE, 3,7-DIMETHYL-, (E)- | C15H24 | 204 | Sesquiterpene |
| 27 | 15.267 | 54599 | 0.35 | 1,6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-8- | C10H16 | 136 | Monoterpene |
| 28 | 15.452 | 94349 | 0.60 | GERMACRENE B | C15H24 | 204 | Sesquiterpene |
| 29 | 15.521 | 184967 | 1.18 | Phenol, 3,5-bis(1,1-dimethylethyl)- | C15H24 | 204 | Sesquiterpene |
| 30 | 15.964 | 33136 | 0.21 | Benzene, 1,2,3-trimethoxy-5-(1-propenyl)-, (E)- | C14H22O | 206 | Aromatic |
| 31 | 16.165 | 32592 | 0.21 | 6,9-PENTADECADIEN-4-OL, 3-BROMO-, [S-[R*,S*-(Z,Z) | C10H17Br | 217 | Monoterpene |
| 32 | 16.380 | 25911 | 0.17 | 3H-PYRAZOLE, 4-(3,5-DIHYDRO-3,3,5,5-TETRAMETH | C15H27BrO | 302 | Sesquiterpene |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 33 | 16.498 | 117381 | 0.75 | DIETHYL PHTHALATE | C12H14O4 | 248 | Ester |
| 34 | 17.220 | 32121 | 0.21 | Cyclohexane, bromo- | C6H11Br | 163 | Reactive Halide |
| 35 | 19.153 | 29582 | 0.19 | 2,3,4-Trimethyl-1-pentanol | C8H18O | 130 | Sesquiterpene |
| 36 | 19.224 | 670502 | 4.28 | Neophytadiene | C20H38 | 278 | Diterpene |
| 37 | 19.478 | 164571 | 1.05 | Neop hyta diene | C20H38 | 278 | Diterpene |
| 38 | 19.673 | 237105 | 1.51 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C20H40O | 296 | Diterpene |
| 39 | 20.521 | 118621 | 0.76 | HEXADECANOIC ACID | C16H32O2 | 256 | Carboxylic |
| 40 | 20.800 | 92596 | 0.59 | HEPTADECANOIC ACID, ETHYL ESTER | C19H38O2 | 298 | Ester |
| 41 | 21.053 | 112208 | 0.72 | 1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10 | C20H34O | 290 | Ether |
| 42 | 21.726 | 102498 | 0.65 | PHOSPHONIC ACID, DIOCTADECYL ESTER | C20H40O | 296 | Ester |
| 44 | 22.669 | 22085 | 0.14 | HEPTADECANOIC ACID, ETHYL ESTER | C19H38O2 | 298 | Ester |
| 45 | 23.133 | 80762 | 0.52 | 1H-Naphtho[2,1-b]pyran-8(4aH)- | C20H32O2 | 304 | Diterpene |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | one, 3-ethenyldecahydro-3 | | | |
| 46 | 23.489 | 30456 | 0.19 | ETHANAMINE, 2,2'-OXYBIS[N,N-DIMETHYL- | C8H20N20 | 160 | Ether |
| 47 | 23.956 | 632535 | 4.04 | Oxandrolone | C19H30O3 | 306 | Ester |
| 48 | 24.778 | 33041 | 0.21 | Undec-10-ynoic acid, 4-methyl-2-pentyl ester | C17H30O2 | 266 | Ester |
| 49 | 24.976 | 213358 | 1.36 | 2,4-PENTADIEN-1-ONE, 4-METHYL-1-(2,3,3-TRIMETHY | C15H22O | 218 | Aromatic |
| 50 | 25.473 | 85464 | 0.55 | 1-(3-DIMETHYLAMINO-PROPYL)-3-HYDROXY-5-(4-M | C21H24N2O4S | 400 | Aromatic ketone |
| 51 | 25.652 | 2092397 | 13.36 | 1,1,4A,7-TETRAMETHYL-2,3,4,4A,5,6,7,8-OCTAHYDRO | C15H26O | 222 | Sesquiterpene |
| 52 | 26.179 | 84642 | 0.54 | 1,2-BENZENEDICARBOXYLIC ACID, DIISOOCTYL ES | C24H38O4 | 390 | Ester |
| 53 | 26.358 | 169567 | 1.08 | 1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4 | C15H26O | 222 | Sesquiterpene |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 54 | 26.562 | 2013324 | 12.86 | 1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTA | C15H26O | 222 | Sesquiterpene |
| 55 | 26.637 | 707775 | 4.52 | 1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTA | C15H26O | 222 | Sesquiterpene |
| 56 | 26.980 | 416149 | 2.66 | Thunbergol | C20H34O | 290 | Diterpenoid |
| 57 | 27.058 | 216163 | 1.38 | Thunbergol | C20H34O | 290 | Diterpenoid |
| 58 | 27.656 | 46428 | 0.30 | Heneicosane | C21H44 | 296 | Ether |
| 59 | 28.259 | 112746 | 0.72 | Methyl-3,4,6-tri-O-methyl-4-O-(methyl-2,3,4-tri-O-methyl.al | C20H36O12 | 468 | Ether |
| 60 | 21.786 | 158662 | 1.01 | Squalene | C30H50 | 410 | Terpene |
| 61 | 32.967 | 119069 | 0.76 | Fumaric acid, 4-heptyl tridecyl ester | C24H44O4 | 396 | Ester |
| 62 | 35.988 | 317001 | 2.02 | RETINAL | C20H28O | 284 | Ketone |
| 63 | 37.518 | 493067 | 3.15 | 3-METHYL-5-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-Y | C15H24O | 220 | Sesquiterpene |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 64 | 41.176 | 192497 | 1.23 | 24-Noroleana-3,12-diene | C29H46 | 394 | Aromatic |
| 65 | 42.823 | 400704 | 2.56 | LUP-20(29)-ENE-3,28-DIOL, (3.BETA.)- | C30H50O2 | 442 | Triterpene |
| 66 | 46.597 | 198341 | 1.27 | 6-epi-shyobunol | C15H26O | 222 | Sesquiterpene |
| | | 1565731 2 | 100. 00 | | | | |

## HPLC Result

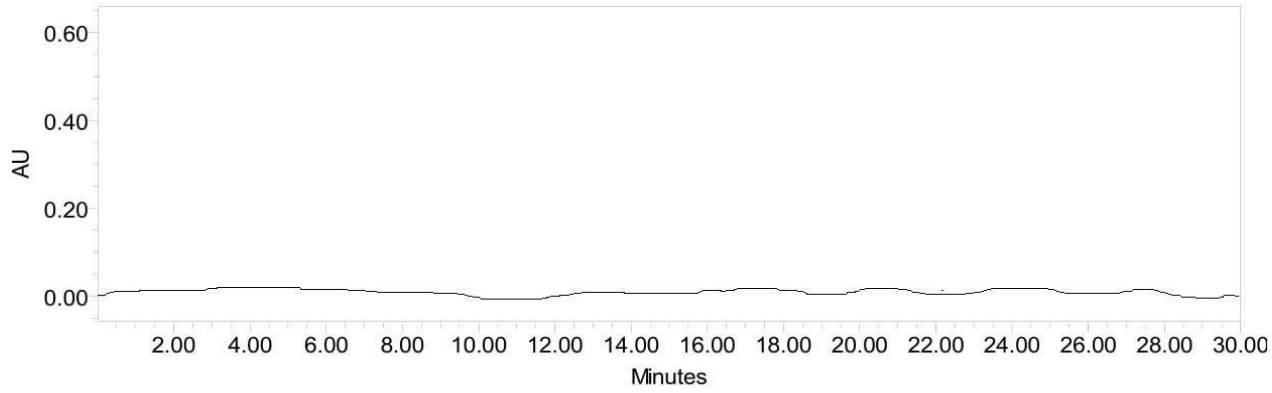


**Figure 12: HPLC chromatogram of Blank**

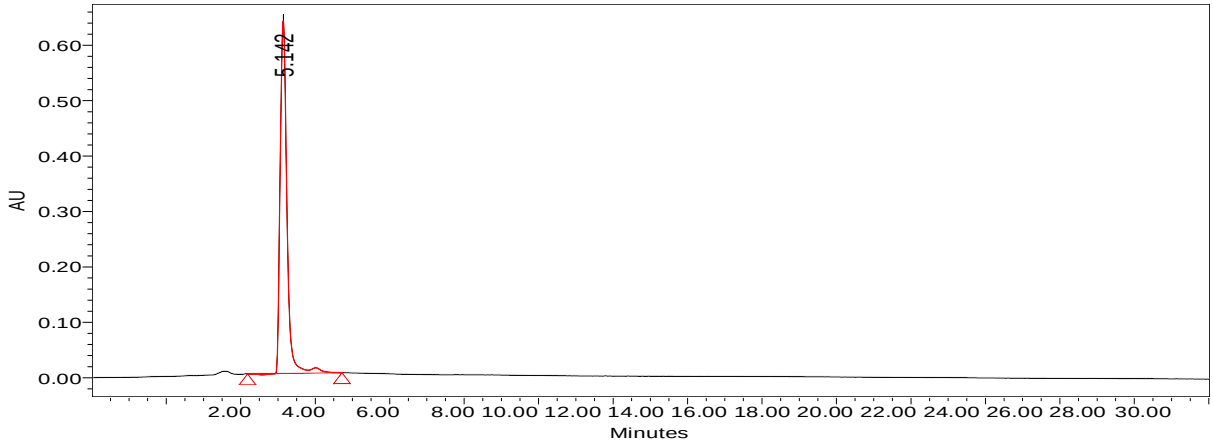


**Figure 13 : HPLC chromatogram of standard of Steviol**

**Table 19 : Analysis of Steviol standard with concentration**

| Retention Time | Area | % Area | Height | % Height | Concentration |
|---|---|---|---|---|---|
| **5.142** | **8122090** | **100.0** | **635642** | **100.0** | **0.2** |

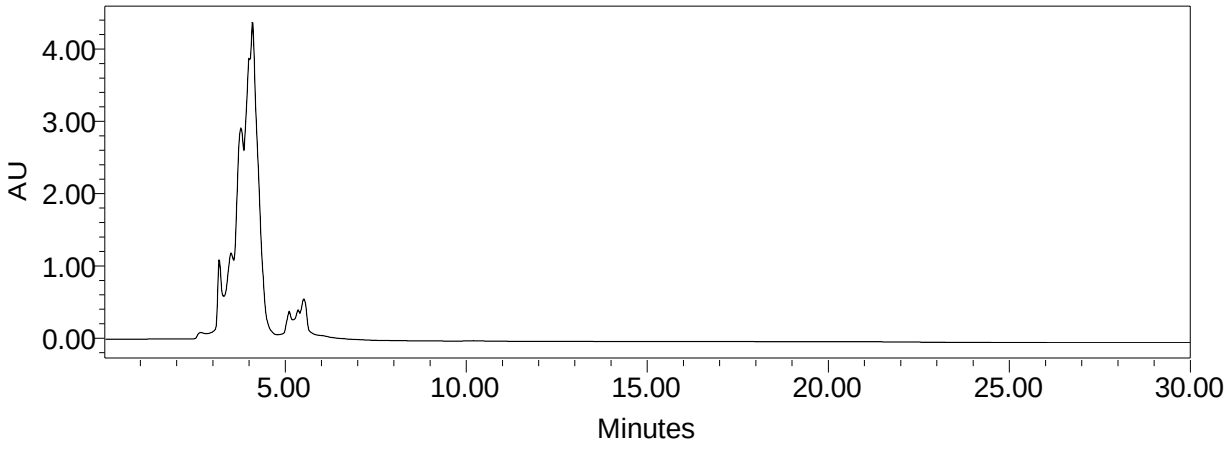


**Figure 14 : HPLC chromatogram of Field grown (FG) extract**

**Table 20 : Analysis of Field grown extract with concentration**

| Retention Time | Area | % Area | Height | % Height | Concentration |
|---|---|---|---|---|---|
| **2.372** | **343820** | **5.89** | **32597** | **11.19** | **0.5287** |

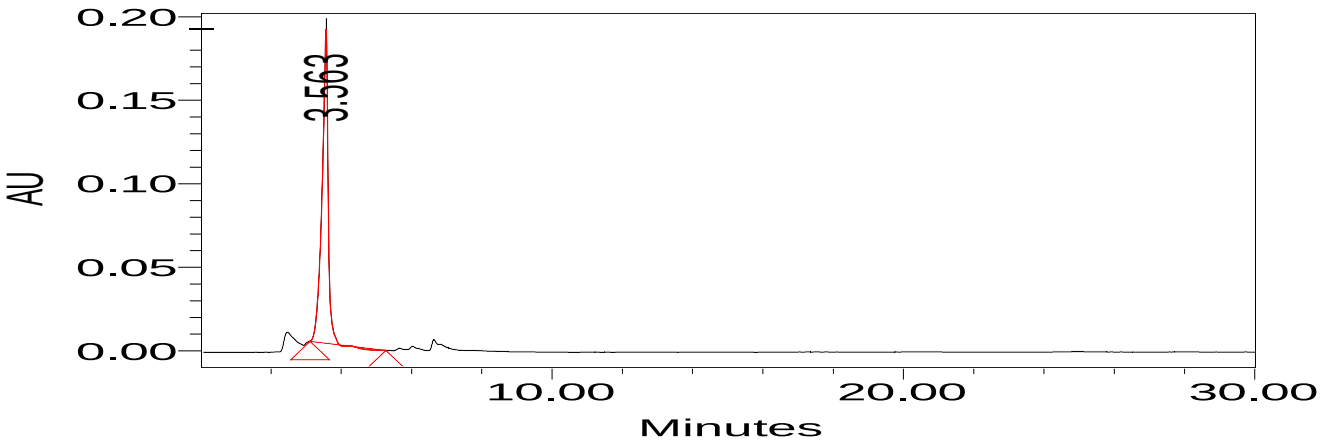


**Figure 15 : HPLC chromatogram of In-vitro grown (IG) extract**

**Table 21 : Analysis of In-vitro grown extract with concentration**

| Retention Time | Area | % Area | Height | % Height | Concentration |
|---|---|---|---|---|---|
| **3.563** | **2334798** | **100.0** | **187052** | **100.0** | **3.5932** |

## 10: DISCUSSION

For the indirect organogenesis of *Stevia rebaudiana* two types of explants sources are used, i.e. internodal segment and the leaf segments were used as cultures that's been maintained at $25 \pm 2^0$ C, with 70 % relative humidity. 16 h day/8 h night photoperiod at a photosynthetic photon flux density of 40 l mol m-2 **s**-1 provided by cool white fluorescent tubes (Philips, India) with sub culturing after every 3- 4 weeks in a plant tissue culture chamber. Best results were shown by leaf segments 100% of the segments were established in the MS media with different concentration of growth regulators as well as 95 % callus induction were reported.

GCMS carried out the chemical profiling of plants called Stevia rebaudiana produces, as its main secondary metabolite diterpene glycosides (steviol) which are natural sweeteners. As a sweetener it advantage that as a terpene it doesn't cause allergic reaction unlike most peptide sweeteners. The presence of chlorogenic acids and flavonoid glycosides in stevia leaves gives the plant additional health benefits, and it could as well affect its organoleptic properties. Identification of the compounds was achieved by using NSIT and Wiley Libraries and Comparisons of retention time.

Lipids and Volatile terpenes were determined by subjecting non polar solvent extracts of stevia leaves to GCMS. *Stevia rebaudiana* plants that mainly contained the sesquiterpenes – betacaryophyllene, trans beta farnesense, alpha humulene, delta cadinene, caryophyllene oxide, nerolidol and an unidentified alcohol. There were a few monoterpenes: linalool, terinen-4-oland alpha terpineol.[10] Main components were Caryophyllene oxide and Spathulenol, making up 43% of the overall content. Signal et al [11] studied the makeup of the essential oil of the aerial parts of five different Stevia rebaudiana genotypes from Brazil and Paraguay cultivated in the coastal region of Tuscany (Italy). In the investigated extract, the main components were diterpenes, of which the most abundant was austro inulin. The other labdanic

diterpene represented in a high level was phenol. Significant portions of the contents of non-polar components belonged to hydrocarbons n-tetracosane and n-pentacosane.

So far no report is available where comparison of between metabolites profiling in in-vitro shoots and field grown shoots of Stevia was carried out. So this GCMS analysis gives us clear idea that these in-vitro shoots are good alternative of field grown shoots which could meet the industrial demand.

HPLC was carried out for the quantification of secondary metabolites in steviol glycoside. The concentration of the plant extract was correlated with the standard of the steviol. The sample was obtained from field grown plants and in-vitro grown plants of *Stevia rebaudiana*. The conditions provided to the In-vitro plants effect the biosynthesis and accumulation of steviol in the respective tissues of the plant as reported by D.Bergs et al[45]. The amount notified in field grown samples of stevia rebaudiana which is 0.004 mg/ml. In the estimated samples in the mentioned study carried out at JUIT plant tissue culture lab has 125 folds higher concentration than the reported samples.

## 11: CONCLUSION

The present study optimized the culture conditions for the production of tissue culture shoots of *Stevi*a within two months duration which can be quality wise comparable to one year field grown plants. Through GC analysis, the study helped in exploring the metabolites concentration in field grown plants and In-vitro grown plants which confirms us that optimized culture conditions are beneficial for the metabolite accumulation and biosynthesis. HPLC was used for estimating the concentration of steviol glycosides, to help us to conclude the key marker compound in both field grown and in-vitro plant extract. This study has reported for the first time about the concentration present in Field grown plant extract was 0.5287 mg/ml and for In-vitro plant extract the concentration is 3.5932 mg/ml of steviol glycoside. Hence these culture conditions provided in the laboratory can be utilized for commercial purposes like bulk production of natural sweetener in food entities.

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