GCMS Analysis of Leaf and Salt Stress Callus of Eggplant (Solanum melongena L.)

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Authors’ contributions

This study was carried out in collaboration between all authors. Author KK designed the study, wrote the protocol and corrected the manuscript. Authors AV and VC managed the literature searches, and performed the plant tissue culture experiment, preliminary phytochemical screening and GC-MS analysis. Author RP wrote the first draft of the manuscript. Author YSJ collected the literature and helped in the manuscript preparation. All authors read and approved the final manuscript.

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Abstract

In the present study, the leaf explant cultured on a MS medium supplemented with BAP (4.44 µM) + NAA (0.98 µM) had the ability to induce more amount of green and friable callus. The 40 days old best grown callus was sub cultured on the combination of various concentrations of BAP (2.22, 4.44, 6.66, 8.88 and 11.1 µM) along with same concentrations of NAA (0.98 µM) with different concentrations of NaCl (20, 40, 60, 80 and 100 mM). The highest percentage of callus was observed on MS + BAP (4.44 µM) + NAA (0.98 µM) + NaCl 20 mM with the percentage of 62.33±1.35. The shoot induction was observed on the same media composition with 95%
response. Preliminary phytochemical analysis of ethanol and methanol extracts of salt callus showed the presence of alkaloids, saponins, steroids, tannins/phenolics, flavonoids, glycosides and reducing sugar. GCMS analysis revealed the presence of 27 compounds in the ethanolic extract of *S. melongena*. Among that there are seven major peaks which indicating the presence of seven major phytochemical constituents. From the twenty seven compounds identified, the most prevailing compounds were, Tetracontane (12.64%), Lupeol (12.44%), N-Tetratetracontane (11.60%), Tetrapentacontane (11.05%) Dotriacontane (10.90%), n-Hexatriacontane (9.18%) and Eicosane (5.26%). Twenty five compounds were identified through GCMS analysis of methanolic salt callus extracts of *S. melongena*. The most prevailing compounds were 1-Heptacosanol (54.39%), Ergosta-5, 7, 22-trien-3-ol (8.37%), Tetracosane (5.26%), Tetratetracontane (4.49%) and Beta Carotene (4.25%).

Keywords: N-Tetratetracontane; tetracontane; reducing sugar; n-Hexatriacontane; n-Hexatriacontane.

1. INTRODUCTION

Salinity is a major problem that affects the growth and productivity of vegetable crops in salt-affected areas. Worldwide around 20% of cultivated land and 33% of irrigated agricultural lands are affected by high salinity [1]. It creates harmful effects to the plants by altering physiological, biological and molecular level. All over the World, almost 25% of the cultivable land has more quantities of salt, mostly NaCl [2]. The salt in the soil delays the growth and water absorption of the plant.

Synthesis of secondary metabolites in plants was reported to be regulated and mediated by environmental factors like drought, light intensity and salt stress. The concentrations of various secondary plant products are strongly dependent on the growing conditions, especially under stress conditions. According to Agoreyo et al. [3] the phytochemical analysis showed that phytate, oxalate, alkaloid and tannin were higher in the round variety of *S. melongena*. Saponin was higher in the oval variety of brinjal. Alkaloids, tannins and saponins have been reported to have medicinal properties. The presence of these phytochemical constituents showed that the *S. melongena* varieties have medicinal property. Sofowora [4] reported the roles of these phytochemicals as analgesic, antiinflammatory, antihypertensive and anti-microbial. Saponins and tannins also exhibit cytotoxic effects and growth inhibition making them suitable as tumor inhibiting agents [5,6]. Proline play an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity [7]. Proline occurs widely in higher plants and accumulation in large amounts than other amino acids. Free proline increased exponentially with the increase in NaCl levels in *S. Nigrum* [8]. The NaCl stress enhanced the total alkaloid content in *S. nigrum* [9,8]. The effect of salt stress on *S. melongena* has been reported by few researchers [8,10].

Plant tissue culture provides a lot of opportunities for plant propagation, plant improvement and production of plants with desirable agronomical characters. Production of virus free, salinity tolerant, disease resistant, herbicide resistant, frost resistant plant are possible through this techniques [11]. It also offers a means of rapid selection on a mass scale and useful for the development of hybrid for salinity resistant yields [12].

The members of the family Solanaceae has about 90 genera and 2,800-3,000 species. The largest genus is *Solanum*. It has around 1,400 species. The Solanaceae is a cosmopolitan family, occurring in tropical and temperate regions throughout the World. Brinjal or eggplant (*Solanum melongena* L.) is an important Solanaceous crop of sub-tropics and tropics. Understanding the importance of salt tolerance in crop plants such as brinjal, the present work is to focus on the production of salinity tolerance brinjal plant through *in vitro* culture technology and to find out the bioactive compounds found in the ethanol and methanol extracts obtained from *in vitro* salt callus through GC-MS analysis.

2. MATERIALS AND METHODS

2.1 Plant Material

The seeds of *Solanum melongena* L. variety Co2 (Solanaceae - Potato family) were obtained from Tamilnadu Agricultural University, Coimbatore Tamilnadu. The seeds were germinated and maintained in earthen pots in shade house at Government Arts College, Coimbatore (Latitude – 11.0168° and longitude – 76.9558°).
2.2 Explants Selection and Mode of Sterilization

The explants such as node, internode and leaves were collected from the shade house grown plants and washed with running tap water for 15 min. The explants were then cut (1-2 cm) separately and washed with Tween 20 detergent solution (5% v/v) for 5 min. After thorough washing, the surface disinfestation of explants were carried out by immersing in 70% ethanol for 30 sec and finally treated with mercuric chloride (0.12 % w/v) (HgCl₂) for 3 min followed by abundant rinsing in with sterile distilled water three to four times to remove trace of toxic chemicals.

2.3 Culture Medium and Culture Conditions

A culture medium containing Murashige and Skoog [13] salts supplemented with macro elements, micro elements and 3% w/v sucrose (Hi Media, India) and solidified with agar 0.8 % (Hi Media, India) was used as a basal medium along with plant growth regulators. The concentrations of NaCl (0, 20, 40, 60, 80 and 100 mM) were prepared for abiotic stress callus induction. The pH of the medium was adjusted to 5.8 with 1N NaOH or 1N HCl. The media were steam sterilized in an autoclave under 15 psi and 121ºC for 20 min. All the cultures were incubated under 50 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) light provided by cool-white fluorescent lamps for 16 h photo period at temperature 24 ± 2ºC.

2.4 Callus Initiation and Shoot Proliferation

Leaf explants were inoculated in the basal medium of Murashige and Skoog (MS) supplemented with 30g/l sucrose and 3% (w/v) agar enriched with cytokinin BAP (2.22 to 11.11 \( \mu \text{M} \)) with NAA (0.98 \( \mu \text{M} \)) or TDZ (1.81 \( \mu \text{M} \)) or Kinetin (2.32 \( \mu \text{M} \)) or 2, 4-D (2.26\( \mu \text{M} \)) containing the various concentrations of NaCl (0, 20, 40, 60, 80 and 100 mM). Callus culture was maintained on MS medium, subcultured every 20 days at 28 ± 2°C with a photoperiod of 16 h. Callus culture was harvested at day 30 of cultivation and dried at room temperature. Twenty explants were used for each treatment. Days for callus induction, the percentage of explants responding for callus induction, the morphology of callus and shoot formation were recorded. In the subsequent sub cultures, the callus obtained in vitro cultures were harvested and used as explants. Sub culturing was carried out at the regular interval of 15-20 days.

2.5 GCMS Analysis

The GC – MS analysis was carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 \( \mu \text{m} \) DF of capillary column. The instrument was set to an initial temperature of 110ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280ºC, at the rate of an increase of 5ºC /min, and maintained for 9 min. Injection port temperature was ensured as 200ºC and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Version –Year 2011 were used MS data library and comparing the spectrum obtained through GC – MS compounds present in the plants sample were identified.

3. RESULTS

3.1 Callus Culture

The morphogenetic potential of leaf explants on MS medium augmented with various concentration BAP alone or BAP with TDZ or KIN or NAA or 2, 4-D are shown in Table 1. Callus initiation was started from 7th day of culture and more amount of callus was formed within 30-35 days. Among the various concentration used for callus induction, 1.0 \( \mu \text{M} \) BAP and 0.2 \( \mu \text{M} \) NAA showed the higher percentage (99.33 ± 1.70) followed by 4.44 \( \mu \text{M} \) BAP and 0.98 \( \mu \text{M} \) NAA (99.33 ± 1.70). The morphology of the callus is friable, dark green and nodular in nature (Fig. 1).

3.2 In vitro Salt Treatment

The 40 days old best grown callus from MS + BAP (2.22 \( \mu \text{M} \)) + NAA (0.98 \( \mu \text{M} \)) was subcultured on the combination of various concentrations of BAP (2.22 \( \mu \text{M} \)) along with same concentrations of NAA (0.98 \( \mu \text{M} \)) with different concentrations of NaCl (20 to 100 mM).
The highest percentage of callus was observed on MS + BAP (2.22 µM) + NAA (0.98 µM) + NaCl 20 mM, with the percentage of 62.33±1.35 (Fig. 1, Table 2). The callus production was very in the medium containing NaCl and compared with normal medium. It was observed that increasing in NaCl concentration decreases the callus production (Table 2). The addition of NaCl at 60 mM to culture medium caused an increase in calli necrosis. No callus formation was observed at the concentration of 80 and 100 mM NaCl. The first callus necrosis was observed at 40 mM NaCl. The higher concentrations of NaCl caused brown coloration and apparent necrosis and reduced callus growth.

Table 1. Effect of MS medium and different concentration and combination of BAP, TDZ, KN, NAA and 2, 4- D on callus induction in leaf explant of Solanum melongena

| S. no | BAP (µM) | TDZ (µM) | KIN (µM) | NAA (µM) | 2,4-D (µM) | Days taken for initiation | % Explant Callus | Callus amount | Nature of the callus |
|-------|----------|----------|----------|----------|------------|--------------------------|-----------------|--------------|---------------------|
| 1     | 2.22     | -        | -        | -        | -          | 13                       | 51.16±1.35      | ++           | WF                  |
| 2     | 4.44     | -        | -        | -        | -          | 14                       | 42.5±1.25       | ++           | WF                  |
| 3     | 6.66     | -        | -        | -        | -          | 15                       | 55.5±1.25       | ++           | WF                  |
| 4     | 8.88     | -        | -        | -        | -          | 14                       | 60.1±1.56       | ++           | WF                  |
| 5     | 11.1     | -        | -        | -        | -          | 16                       | 40.5±1.34       | ++           | WF                  |
| 6     | 2.22     | 1.81     | -        | -        | -          | 14                       | 29.66±1.23      | ++           | WF                  |
| 7     | 4.44     | 1.81     | -        | -        | -          | 12                       | 25.33±1.41      | ++           | WF                  |
| 8     | 6.66     | 1.81     | -        | -        | -          | 13                       | 40.16±1.35      | ++           | WF                  |
| 9     | 8.88     | 1.81     | -        | -        | -          | 10                       | 60.0±1.29       | ++           | WF                  |
| 10    | 11.1     | 1.81     | -        | -        | -          | 11                       | 46±1.18         | ++           | WF                  |
| 11    | 2.22     | 2.32     | -        | -        | -          | 11                       | 73.33±1.28      | ++           | GF                  |
| 12    | 4.44     | 2.32     | -        | -        | -          | 13                       | 80.5±1.33       | + +          | YF                  |
| 13    | 6.66     | 2.32     | -        | -        | -          | 14                       | 85.33±1.54      | + ++         | WF                  |
| 14    | 8.88     | 2.32     | -        | -        | -          | 10                       | 79.66±1.35      | +             | WF                  |
| 15    | 11.1     | 2.32     | -        | -        | -          | 13                       | 69.3±1.33       | +             | YF                  |
| 16    | 2.22     | -        | 0.98     | -        | -          | 11                       | 87.66±1.66      | + ++         | GF                  |
| 17    | 4.44     | -        | 0.98     | -        | 07         | 99.33±1.70                | + ++           | GF           |
| 18    | 6.66     | -        | 0.98     | -        | 08         | 77.33±1.35                | ++           | YF           |
| 19    | 8.88     | -        | 0.98     | -        | 10         | 90.83±1.16                | + + +         | WF           |
| 20    | 11.1     | -        | 0.98     | -        | 12         | 63±1.31                   | ++           | YF           |
| 21    | 2.22     | -        | -        | 2.26     | 11         | 72.5±1.43                 | ++           | GF           |
| 22    | 4.44     | -        | -        | 2.26     | 13         | 79.5±1.33                 | + + +         | YF           |
| 23    | 6.66     | -        | -        | 2.26     | 14         | 84.33±1.35                | + + +         | WF           |
| 24    | 8.88     | -        | -        | 2.26     | 10         | 79.83±1.42                | + + +         | WF           |
| 25    | 11.1     | -        | -        | 2.26     | 13         | 62.33±1.35                | + + +         | GF           |
| Basal medium | - | - | - | - | - | - | - | - | - |

Table 2. Survival percentage and callus morphology scores of BAP and NAA derived callus in the stressed media with different concentration of NaCl

| S. no | BAP (µM) | NAA (µM) | NaCl (mM) | Survival percentage | Callus amount | Nature of the callus | Nature of response |
|-------|----------|----------|-----------|---------------------|---------------|----------------------|-------------------|
| 1     | 4.44     | 0.98     | 0         | 96.83±1.42          | ++            | GF                   | CWMS              |
| 2     | 4.44     | 0.98     | 20        | 62.33±1.35          | ++ +          | GF                   | CWMS              |
| 3     | 4.44     | 0.98     | 40        | 24.33±1.35          | +             | YF                   | BRC               |
| 4     | 4.44     | 0.98     | 60        | 9.83±1.51           | +             | YF                   | BRC               |
| 5     | 4.44     | 0.98     | 80        | -                   | -             | -                    | -                 |
| 6     | 4.44     | 0.98     | 100       | -                   | -             | -                    | -                 |
| Basal medium | - | - | - | - | - | - | - | - |

YF-Yellow Friable, WF-White Friable, GF-Green Friable
WS-Callus With Shoot, CWMS-Callus With Multiple Shoot, CM-Callus Multiplication, BLC-Black Callus, BRC-Brown Callus, CD-Callus Dead, NR- No Response
3.3 Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of ethanolic and methanolic extracts of salt callus derived *Solanum melongena* are presented in Table 3. The phytochemical analysis showed the presence of alkaloids, saponins, steroids, tannins/phenolics, flavonoids, glycosides and reducing sugar. It was concluded that the salt callus extracts of eggplant contains important constituents for pharmacological activities.

3.4 GCMS Analysis

GCMS analysis revealed the presence of 27 and 25 compounds in the ethanolic and methanolic extracts of salt callus of *S. melongena* by comparing their retention time and by interpretation of their mass spectra (Figs. 2 and 3). The ethanolic extract of *S. melongena* shows twenty seven peaks (Fig. 2). Among these there are seven major peaks which indicating the presence of seven major phytochemical constituents. From the twenty seven compounds...
identified, the most prevailing compounds were, Tetracontane (12.64%), Lupeol (12.44%), N-Tetracontane (11.60%), Tetratetracontane (11.05%) Dotriacontane (10.90%), n-Hexatriacontane (9.18%) and Eicosane (5.26%) (Table 4).

Twelve five peaks were observed in GC-MS chromatogram analysis of the methanolic extract of S. melongena (Fig. 3). Among these there are five major peaks which indicating the presence of five major phytochemical constituents. From the twenty five compounds identified, the most prevailing compounds were 1-Heptacosanol (54.39%), Ergosta-5, 7, 22-trien-3-ol (8.37%), Tetracosane (5.26%), Tetratetracontane (4.49%) and Beta Carotene (4.25%). The active principles with their retention time (RT), molecular formula and molecular weight (MW) and peak area in the ethanolic salt callus extract of S. melongena are presented in Table 5.

4. DISCUSSION

In the present study, the explant cultured on medium supplemented with BAP (4.44 µM) + NAA (0.98 µM) had the ability to induce more amount of green and friable callus. Similar observation was reported in Solanum melongena [14], that NAA was involved in the development

## Table 3. Preliminary phytochemical analysis of ethanol and methanol extracts Solanum melongena salt callus

| S. no. | Compounds   | Ethanol extract | Methanol extract |
|-------|-------------|-----------------|-----------------|
| 1     | Alkaloids   | +               | +               |
| 2     | Saponins    | +               | +               |
| 3     | Steroids    | +               | +               |
| 4     | Tannins     | +               | +               |
| 5     | Phenol      | +               | +               |
| 6     | Flavonoids  | +               | +               |
| 7     | Glycosides  | +               | +               |
| 8     | Triterpenoids| +              | +               |
| 9     | Reducing sugars | +       | +               |

+ Denotes presence of compound; - Denotes absence of compound

## Table 4. Phytocomponents identified in ethanolic leaf callus extracts of Solanum melongena by GCMS

| S. no. | RT   | Compound name                                  | Molecular formula | Molecular weight | Peak area (%) |
|-------|------|-----------------------------------------------|-------------------|------------------|---------------|
| 1     | 5.650| Benzeneethanamine                             | C_{22}H_{24}FNO_3 | 381              | 0.00          |
| 2     | 6.208| Methylbenzeneethanamine                        | C_{9}H_{13}N      | 135              | 0.06          |
| 3     | 7.017| Methoxy, Phenyl- ,Oxime                        | C_{8}H_{9}NO_2    | 151              | 1.21          |
| 4     | 14.658|N-Ethylformamide                               | C_{3}H_{7}NO      | 73               | 0.04          |
| 5     | 25.614|Propane, 2-methoxy-2-methyl                    | C_{2}H_{12}O      | 88               | 0.08          |
| 6     | 26.084|4-Undecene                                     | C_{12}H_{24}      | 168              | 0.18          |
| 7     | 26.982|Neophytadiene                                  | C_{20}H_{38}      | 278              | 0.74          |
| 8     | 28.472|1-Hexanol                                      | C_{10}H_{20}O     | 158              | 0.34          |
| 9     | 29.161|2-Isopropyl-5-methyl-1-heptanol                | C_{11}H_{20}O     | 172              | 0.34          |
| 10    | 29.595|Hexadecanoic acid                              | C_{18}H_{36}O_2   | 284              | 1.61          |
| 11    | 29.794|4- (3,5-Di-tert-butyl-4-hydroxyphenyl) butyl acrylate | C_{21}H_{32}O_3   | 332              | 1.26          |
| 12    | 31.282|3,7,11,15-Tetramethylhexadec-2-en-1-ol         | C_{26}H_{40}O     | 296              | 1.09          |
| 13    | 32.265|Palmitic acid                                  | C_{18}H_{36}O_2   | 284              | 1.46          |
| 14    | 33.476|Tetracontane                                   | C_{24}H_{32}      | 618              | 1.87          |
| 15    | 34.686|Eicosane                                       | C_{20}H_{42}      | 282              | 5.26          |
| 16    | 36.108|n-Hexatriacontane                              | C_{30}H_{64}      | 506              | 9.18          |
| 17    | 37.841|Dotriacontane                                  | C_{22}H_{46}      | 450              | 10.90         |
| 18    | 40.009|Tetratetracontane                              | C_{54}H_{110}     | 758              | 11.05         |
| 19    | 42.547|N-Tetratetracontane                            | C_{44}H_{90}      | 618              | 11.60         |
| 20    | 43.201|Squalene                                       | C_{30}H_{50}      | 410              | 1.46          |
| 21    | 43.754|n-Tetracosane                                  | C_{30}H_{50}      | 562              | 0.77          |
| 22    | 44.368|Tetracontane                                   | C_{40}H_{82}      | 562              | 12.64         |
| 23    | 45.358|Hexacosane                                     | C_{60}H_{122}     | 842              | 1.49          |
| 24    | 45.546|1,54-Dibromotetrapentacontane                  | C_{54}H_{108}Br_2 | 914              | 2.13          |
| 25    | 45.986|Tetrapentacontane                              | C_{54}H_{110}     | 758              | 10.12         |
| 26    | 46.439|4,6-Cholestadien-3.beta.-ol                    | C_{27}H_{44}O     | 384              | 0.67          |
| 27    | 46.871|Lupeol                                         | C_{30}H_{50}O     | 68               | 12.44         |
of callus. Some studies revealed that plant hormones are essential for induction of callus from explants and no callus was induced by basal medium without hormones. For shoot proliferation cytokinin is one of the major important factors affecting the response [15]. The effect of BAP on shoot initiation and multiple shoot formation has been demonstrated in many cases using different explants [14,16,17,18]. In the present study also BAP along with NAA produced maximum number of shoots (15.13±1.09). These results were in confirmation with the observation reported by Muthusamy et al. [19] and Rahman et al. [20] against leaf and cotyledon morphogenic response of *S. melongena* varieties respectively.

![Fig. 2. Phytocomponents identified from ethanolic extracts of *Solanum melongena*](image1)

![Fig. 3. Phytocomponents identified from methanolic extracts of *Solanum melongena*](image2)
### Table 5. Phytocomponents identified in methanolic leaf callus extracts of *Solanum melongena* by GCMS

| S. no. | RT    | Compound name                                      | Molecular formula  | Molecular weight | Peak area (%) |
|--------|-------|---------------------------------------------------|--------------------|------------------|---------------|
| 1      | 6.98  | Hexamethylphosphoramide                           | C₆H₁₈N₃OP          | 179              | 0.36          |
| 2      | 8.233 | 3,6-Bis-dimethylaminomethyl-2,7-dihydroxy-fluoren-9-one | C₁₀H₂₂N₂O₂         | 326              | 0.29          |
| 3      | 10.782| Benzeneethanamine                                  | C₂₅H₂₆F₂NO₂Si₂     | 475              | 0.65          |
| 4      | 32.363| Pentanoic acid                                     | C₅H₁₀O            | 172              | 0.23          |
| 5      | 33.507| Decanedioic acid                                   | C₃₀H₅₈O₄           | 482              | 0.42          |
| 6      | 34.715| 2,6,10,15-Tetramethylheptadecane                   | C₂₁H₄₄            | 296              | 1.17          |
| 7      | 36.139| n-Dotriacontane                                    | C₃₂H₆₆            | 450              | 2.35          |
| 8      | 37.874| Tetrapentacontane                                  | C₅₂H₁₁₀           | 758              | 2.97          |
| 9      | 39.225| Undecanecan                                       | C₁₁H₂₂O           | 170              | 0.56          |
| 10     | 40.039| Tetrapentacontane                                  | C₅₂H₁₁₀           | 758              | 2.72          |
| 11     | 42.568| N-Tritriacontane                                   | C₂₃H₆₈            | 464              | 3.38          |
| 12     | 43.225| 2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane | C₁₅H₂₆O          | 222              | 0.44          |
| 13     | 43.787| Heneicosan                                         | C₂₂H₄₆            | 310              | 0.32          |
| 14     | 44.225| Nonyl-Phenol mix of isomers                        | C₁₃H₂₄O           | 220              | 0.37          |
| 15     | 45.750| Cyclobutaneethanol                                  | C₁₃H₁₈O           | 154              | 0.74          |
| 16     | 45.386| N-Tetracosane                                      | C₂₄H₅₀            | 338              | 5.26          |
| 17     | 45.549| Ergosta-5,7-Dien-3-ol                               | C₂₆H₄₆O           | 398              | 8.37          |
| 18     | 44.725| [1,1'-Biphenyl]-4,4'-dicarboxaldehyde              | C₁₁H₁₀O₂          | 210              | 1.76          |
| 19     | 45.062| Heptacosyl heptafluorobutyrate                     | C₂₃H₅₅F₂O₂        | 592              | 54.39         |
| 20     | 45.677| Beta Carotene                                      | C₂₆H₅₆            | 536              | 4.25          |
| 21     | 45.908| Hexahydrofarnesyl acetone                          | C₁₈H₂₆O           | 268              | 2.58          |
| 22     | 46.003| Hexacontane                                       | C₁₉H₁₄O₂          | 842              | 4.49          |
| 23     | 46.436| 7,7,9,9-Tetrakis-hydroxymethyl-1,4dioxa-spiro[4.5]decan-8-ol | C₁₂H₂₂O₇         | 278              | 0.38          |
| 24     | 46.483| 4-Fluoro-2-nitroaniline                            | C₁₈H₂₂FN₅O₃       | 351              | 0.25          |
| 25     | 46.885| d-Norandrostanne                                   | C₁₈H₃₀            | 246              | 1.29          |

Callus formation at the leaf segments of *S. melongena* on MS medium supplemented with a higher concentration of BAP and lower concentration of NAA is the result obtained in *Tylophora indica* [16], *Ceropegia pusilla* [17] and *S. melongena* [21]. The synergistic effect of BAP in combination with an auxin has been demonstrated in *S. melongena* [19,22,21]. Of the different level of BAP tested along with NAA or TDZ or KIN or 2,4D, the BAP (2.22 µM) and NAA (0.98 µM) proved to be most effective, as in this medium an average of 15.13±1.09 shoots per explants developed in 99.33% of culture. Lowering the concentration of BAP a reduction in the number of shoots per culture was recorded. Similarly higher concentration, the number as well as percentage was drastically reduced.

The reduction in the fresh weight of the callus is the indicator of the effect of NaCl on callus induction. Increase in salt concentration with a decrease in fresh weight of the callus previously reported in *Oryza sativa* [23]. The fresh weight reduction might be due to the decrease in water availability by the increased NaCl concentration. The callus was exposed to regeneration medium with different concentration of NaCl for analyzing the effects of salinity on plant regeneration. The regeneration frequency decreased with increasing NaCl concentration. In *Solanum nigrum* also the increasing concentration of NaCl reduces the regeneration capacity [8]. There was a gradual decrease in root length than the shoot length as the NaCl concentration increases. This may be due to the excess soluble salts leads to osmotic stress [24].

In addition to major compounds there are some minor peak compounds also has some biological active principles like 10-n-Hexadecanoic acid, is...
used as an anti-inflammatory compound. The chemical 4- (3, 5-Di-tert-butyl-4-hydroxyphenyl) butyl acrylate used as an ethanomedicinal and antimicrobial because butyl acrylate and their derivatives are used pharmaceutically as anticancer drugs [25]. Another compound 3,7,11,15-Tetramethylhexadec-2-en-1-ol used as an anti-tuberculosis, insecticidal, anti-inflammatory, antioxidant, antimicrobial [26]. The squalene were also has anticancer, anti-oxidant, chemopreventive, pesticide, anti-tumor, and sunscreen properties [27(33)].

Among the twenty five peaks observed in GC-MS chromatogram analysis of the methanolic extract of S. melongena, five major peak compounds all of them were biologically active compounds. 1Heptacosanol have nematicidal, anticancer, antioxidant and antimicrobial activity [26(32)] followed by Ergosta-5, 7, 22-trien-3-ol were also have antimicrobial activity [28(34)]. Tetracosane showed significant cytotoxicity colon cancer cells. It also showed some toxicity against the estrogen dependent breast cancer, a variety of pharmacological activities, including other cytotoxicity [29,30]. Tetratetracontane, Neutral components, which has medicinal importance as an anti-inflammatory, antibacterial, antiulcer genic [31]. Beta carotene have been reported to protecting against cancer, antioxidant and cardiovascular diseases [32]. The minor peak compounds also has some biological active principles, lupeol was reported to have antiprotazoal, antimicrobial, anti-inflammatory, antitumor and chemo preventive properties [33]. Tetratetracontane, Neutral components in the leaves and seeds of Syzygium cumini, the plant which has medicinal importance as an anti-inflammatory, antibacterial, antiulcergeni [31]. Eicosanoid has the antitumor activity against the human gastric cell line [34].

The presence of various bioactive compounds in the both Ethanolic and methanolic extracts of salt stressed callus of S. melongena justifies that, the salt stress have been induced to produce strong bioactive compounds. However, isolation of individual phytochemical constituents and subjecting it to the biological activity from salinity tolerance callus will definitely give fruitful results. The results, shows that the salt stressed S. melongena callus contains various bioactive compounds. Therefore, it is concluded that in vitro clonal propagation with salt stress could alter the biochemical changes which could be a phytopharmaceutically and morphopotentially importance.

5. CONCLUSION
Preliminary phytochemical analysis of ethanolic and methanolic extracts of salt callus derived Solanum melongena showed the presence of alkaloids, saponins, steroids, tannins/ phenolics, flavonoids, glycosides and reducing sugar presence of these phytochemical constituents showed that the S. melongena have medicinal property.

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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