# *IN VITRO* PRODUCTION OF NOVEL VOLATILE COMPOUNDS FROM *FOENICULUM VULGARE* MILL.CELL LINES

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**Abstract**

The idea to get desired quality and quantity of volatile compounds by altering the amount and concentration of PGRs during *in vitro* growth of a plant is considered as one of the most advanced ones, in modern bioscience technologies. During the present piece of work, callogenesis (81 %), somatic embryogenesis (92 %) and regeneration (90 %) on MS basal medium, supplemented with 2, 4-D (3.0 mg/L) from *Foeniculum vulgare* Mill.'s leaf explants. Identification of volatile compounds during *in vitro* cell lines growth of fennel was done using Gas Chromatography Mass Spectrophotometer (GC/MS). Sixty five compounds were identified during callogenesis, out of which two were found to be novel. One hundred and twenty compounds were detected during somatic embryogenesis, out of which nine were novel. Similarly, one hundred compounds were found during regeneration out of which three were identified as the novel ones. It is noteworthy that above mentioned experimental technique to produce desired quantity and quality of novel/already cited compounds by manipulating various PGRs during *in vitro* growth of a plant, can be utilized in future for commercial purposes on a larger scale in pharmaceutical industry.

**Keywords**: *Foeniculum vulgare* Mill, GC/MS, New volatile compound, PGRs

## Introduction

*Foeniculum vulgare* Mill. generally called fennel, and locally known as saunf, is a perennial plants with aromatic odor. It belongs to Apiaceae family, originated from Mediterranean region and Southern Europe (Fiore *et al*., 2012). Fennel seeds contain many phytochemicals like polyacetylenes (falcarinol, falcarindiol, and falcarindiol-3-acetate), polyphenols (caffeic acid, gallic acid, apigenin-7-o-glucoside, ferulic acid, syringic acid, isovitexin, phloridzin), Flavonoids (quercetin and arabinosid), phenylpropanoid derivatives and monoterpenoids (Harborne *et al*., 1971, 1972, 1984; Renjie *et al*., 2010).

Many researcher have already paid attention to the idea of bioactive compound's production during *in vitro* growth of a plant, e.g., Lyer *et al*. (2009) established embryogenic culture system in nutmeg for the *in vitro* production of α and β-pinene, sabinene, myristicin, safrole and methyl eugenol etc. Similarly, Nagar and Mehwaki (2014) got trans-Anethol from two tissue cultured fennel genotypes. Saljooghianpour and Javaran (2013) identified phytochemical components including p-Xylene, Tetradecane, tridecane, Oleic acid and Hexadecane etc. from *in vitro* grown Aloe plantlets by gas chromatography-mass spectrometry (GC/MS).

In a nutshell, aim of current study was to extend a fast and reproducible process for the isolation and identification of novel as well as already reported volatile compounds from fennel during its *in vitro* growth using GC/MS technique. This study may help to produce desired quality and quantity of novel volatile compounds from medicinal plants, using different PGRs during their *in vitro* growth.

## Materials and Methods

***In vitro* growth of *Foeniculum vulgare* Mill. cell lines:** *Foeniculum vulgare* plants were grown from sterilized seeds, certified by Punjab Seed Corporation. The leaf, nodal and intermodal explants were washed with tap water using few drops of liquid detergent and then dipped in 5 % commercial sodium hypochlorite. Culture media was sterilized in autoclave at 121 °C for 15 lb/In$^2$; The explants were inoculated on MS media supplemented with different concentration of 2,4-D. Periodically i:e after every 15 days, *in vitro* cultures were sub-cultured on same medium. Observations regarding the response of *in vitro* growth were made weekly. Data of callogenesis, somatic embryogenesis and regeneration (%) was also recorded.

**Detection and identification of novel/ already cited volatile compounds from *in vitro* cell lines of *Foeniculum vulgare* Mill, using GC/MS:** Plant material i.e. callus, somatic embryos and regenerated tissues were cut into small pieces, and grounded to powder form. One or two grams of each of these samples were soaked in 10 ml of 95% pure n-hexane for 3 days, shaken on incubator shaker strongly every day and filtered, twice. For complete extraction of essential oils process was repeated for a total of 5 times. Finally each sample type was mixed, filtered and evaporated to concentrate and stored at 4°C in dark until analysis.

These volatile constituents were analyzed by GC/MS. The Injection temperatures of GC/MS was kept at 250 °C and 200 ºC, interfaced with Agilent and QP detectors correspondingly. The QP 2010 chromatograph, (Ionization voltage 70 eV, m/z scan range 55-950 Da,), equipped with a DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm) was used. The oven temperature was held at 05 °C for 01 min, then programmed from 50-100 °C at a rate of 05 °C/min, held again for 01 min, then increased up to 200 °C at the rate 10 °C/min and kept at the final temperature for 05 min, using Helium as a carrier gas. The percentage composition of volatile compounds was computed from GC peak areas. Qualitative analysis was based on a comparison of retention times, indices and mass spectra with the corresponding data in the literature (NIST Standard Reference Database 1A, 2014).

## Results

**Callogenesis, somatic embryogenesis and regeneration of *Foeniculum vulgare* Mill. with different concentrations of 2, 4-D (mg/L) in MS basal medium:** Response of different explants *i.e.* leaf, internode and node on MS medium (Murashige and Skoog, 1962) supplemented with different concentrations of 2, 4-D (mg/L) was recorded. Leaf explants of fennel showed maximum callogenesis (81 %), somatic embryogenesis (92 %) and regeneration (90 %) with 2, 4-D (3.0 mg/L) on MS basal medium (Figs 1.A, 1.B and 1.C and Table 1).

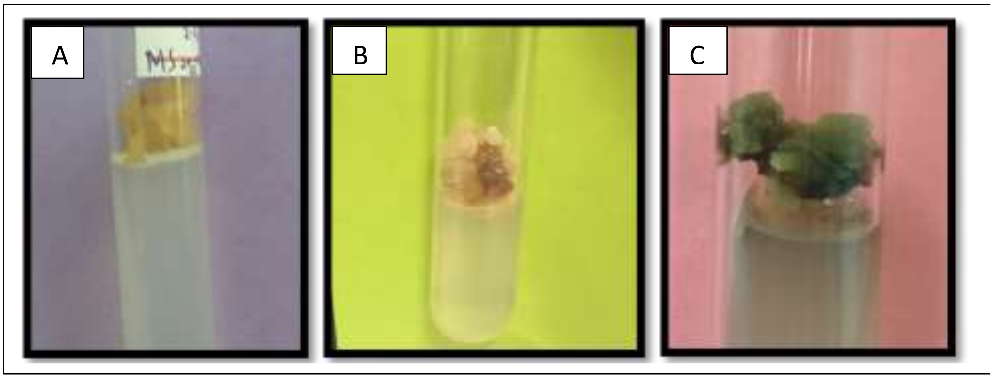


Fig 1. (A). Callogenesis from the Leaf explants of *Foeniculum vulgare* Mill. after 15 days of inoculation on MS basal medium supplemented with 2, 4-D (3.0 mg/L). (B). Somatic embryogenesis from the Leaf explants of *Foeniculum vulgare* Mill. after 30 days of inoculation on MS basal medium supplemented with 2, 4-D (3.0 mg/L). (C). Regeneration from the leaf explants of *Foeniculum vulgare* after 45-days of inoculation on MS basal medium supplemented with 2,4- D (3.0 mg/L).

**Table 1. Callogenesis, somatic embryogenesis and regeneration of *Foeniculum vulgare* Mill. with different concentrations of 2, 4-D (mg/L) in MS basal medium using leaf explants**

| 2,4-D (mg/L) used | Callogenesis (%mean) | Somatic embryogenesis (% mean) | Regeneration (% mean) | LSD Value |
|---|---|---|---|---|
| **3.0** | **$81^{a}$**± 0.71 | **$92^{a}$**± 0.11 | **$90^{a}$**± 0.47 | 0.99 |
| 2.5 | $58^{c}$ ± 0.47 | $80^{ab}$± 0.25 | $67^{b}$± 0.51 | |
| 2.0 | $69^{b}$± 0.11 | $73^{c}$± 0.47 | $73^{c}$± 0.77 | |

± Standard deviation of the mean;

No. Cultures used for each treatment: 50

**Identification of volatile compounds produced during Callogenesis, Somatic Embryogenesis and Regeneration of *Foeniculum vulgare* Mill. using GC/MS**

**i. Volatile compounds isolated during callogenesis on MS Basal medium supplemented with 2, 4-D (1.5 to 2.5mg/L) from leaf explants of *Foeniculum vulgare* Mill. using GC/MS:** Sixty five compounds were isolated from callogenesis of fennel (Table 2 and Fig 2.A) using GC/MS, out of which two (1-iodoundecane $$ Undecane, 1-iodo- $$ and 2-Bromononane $$ sec-Nonyl bromide $$ Nonane, 2-Bromo-$$) were found to be novel using Mass Spectral Deconvolution and Identification System (Mallard and Reed, 1997).

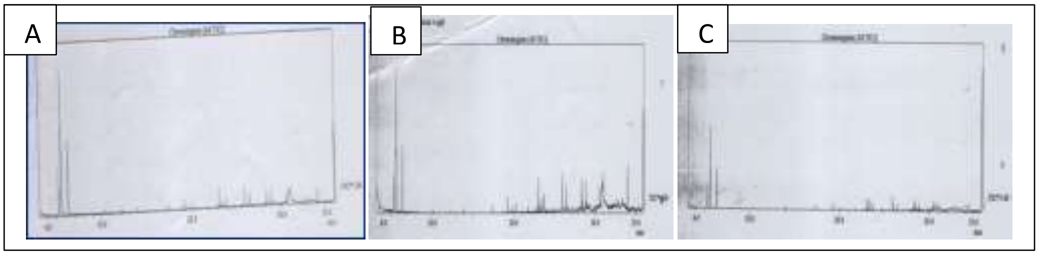


Fig 2. (A.) The chromatogram of GC/MS showing the isolation of volatile compounds during callogenesis of fennel. (B). The chromatogram of GC/MS showing the isolation of volatile compounds during somatic embryogenesis of fennel. (C). The chromatogram of GC/MS showing the isolation of volatile compounds during regeneration of fennel.

**Table.2.Volatile compounds isolated during callogenesis on MS Basal medium supplemented with 2, 4-D (1.5 to 2.5mg/L) from leaf explants of *Foeniculum vulgare* Mill. using GC-MS:**

| Retention Time (min) | Volatile compounds isolated during callogenesis |
|---|---|
| **5.258** | Ethylbenzene $$ Benzene, ethyl- $$ Ethylbenzol $$ EB $$ Phenylethane $$Aethylbenzol $$ Ethylbenzene $$ Etilbenz. Ethylbenzene. Ethylbenzene. Ethylbenzene. Hydroxylamine,O-(phenylmethyl)-$$ Hydroxylamine, O- benzyl- $$(Benzyloxy)amine $$ O- Benzylhydroxylamine$$. |
| **5.550** | o-Xylene. o-Xylene. p-xylene. Benzene, 1,3 –dimethyl-. p- Xylene. |
| **6.325** | o-Xylene. P-Xylene. o-Xylene. p-xylene. ethylbenzene. |
| **17.375** | 1H-Tetrazol- 5- amine. 5H-Tetrazol- 5- amine $$5H- Tetrazol-5-amine # $$. 1- 1odoundecane $$ Undecane, 1- iodo- $$. 2,2- Dimethylpropanoic anhydride $$Trimethylacetic anhydride $$Pivalic anhydride $$ Pivalic anhydride $$ propanoic. 3-Hexanonane, 2,2- dimethyl |
| **19.242** | Nonane, 1- iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1- n- Nonyl iodide $$ 1- Iodononane $$. Butane 2,2- Dimethyl-. Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3$$ UN 1208 $$. Butane, 2,2-dimethyl-. 4-Heptanone,3-methyl-$$ 3-Methyl-4-heptanone$$. |
| **19.242** | Nonane, 1- iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1- n- Nonyl iodide $$ 1- Iodononane $$. Butane 2,2- Dimethyl-. Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3$$ UN 1208 $$. Butane, 2,2-dimethyl-. 4-Heptanone,3-methyl-$$ 3-Methyl-4-heptanone$$. |
| **23.108** | Hexane, 3,3 – Dimethyl, Nonane, 1- iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1- n- Nonyl iodide $$ 1- Iodononane $$. Hexane, 3,3 – Dimethyl-$$ 3,3-Dimethylhexane $$. Heptane, 3,3-dimethyl-. 2-Bromononane, $$ sec-Nonyl bromide $$ Nonane, 2-Bromo- $$. |
| **23.450** | Pentanoic acid, 5-hydroxy-, 2,4-di-t-butyiphenyl esters $$ 2,4-Ditert- butylphenyl 5-hydroxypentanoate. #$$. Pentanedioic acid, (2,4-di-t-butylphenyl) mono-ester$$ 5-(2,4-Ditert- |

| | |
|---|---|
| | butylphenoxy)-5-oxopentanoic acid # $$. Phenol,2,4-bis(1,1-dimethylethyl)- $$ Phenol, 2,4-di-tert-butyl-$$ 2,4-Di-tert-butylphenol $$ 2,4-di-t-Butylphenol $$. Phenol, 3,5-bis(1,1-Dimethylethyl)-$$ Phenol, 3,5- di-tert-butyl-$$ 3,5-DI-tert-butylphenol $$ Phenol, 3,5-bis(t-butyl)-. Phenol,2,4-bis(1,1-dimethylethyl)- |
| **23.775** | Nonane, 1- iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1- n- Nonyl iodide $$ 1- Iodononane $$. 2-Bromononane, $$ sec-Nonyl bromide $$ Nonane, 2-Bromo- $$. Hexane, 3,3 – Dimethyl-. Nonane,1-iodo-. Nonane, 5-methyl-5-propyl- $$ 5-Methyl-5-propylnonane #$$. |
| **25.967** | Hexane, 3,3 – Dimethyl-. Nonane, 1- iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1- n- Nonyl iodide $$ 1- Iodononane $$. 3,3-Dimethylhexane $$. Hexane, 3,3 – Dimethyl-$$ 3,3-Dimethylhexane $$. Heptane, 3,3-dimethyl-. Dodecane, 1- iodo-. |
| **26.500** | Nonane, 1- iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1- n- Nonyl iodide $$ 1- Iodononane $$. 2-Bromononane, $$ sec-Nonyl bromide $$ Nonane, 2-Bromo- $$. Hexane, 3,3 – Dimethyl-. Nonane,1-iodo-. Decane, 1- iodo-. |
| **28.400** | Nonane, 1- iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1- n- Nonyl iodide $$ 1- Iodononane $$. Dodecane, 1- iodo-. Hexane, 3,3 – Dimethyl-. 3- Ethyl-3-methylheptane $$ Heptane, 3-ethyl-3-methyl-$$. Decane, 1- iodo-. |
| **35.925** | 1,2-benzenedicarboxylic acid, diisooctyl ester. Di—n-octyl phthalate. 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester$$ Mono(2- ethylhexyl) phthalate$$ Phthalic acid, mono-(2-et). 1,2-benzenedicarboxylic acid, diisooctyl ester $$ Diiooctylphthalate $$ Hexaplas M/O $$ Isooctyl phthalate $$ Corfl. Bis(2-ethyhexyl)phthalate $$ 1,2- benzenedicarboxylic acid, bis(2-Ethylexyl) ester $$ Phthalic acid, bis(2-ethylhexyl). |

**ii. Volatile compounds identified during somatic embryogenesis on MS basal medium supplemented with 2, 4-D (3.0mg/L). from leaf explants of *Foeniculum vulgare* Mill. using GC/MS:** One hundred and twenty compounds were isolated during somatic embryogenesis of fennel, out of which nine were novel (Table 3 and Fig 2.B). The novel compound isolated were ethanone, 1-cyclopropyl 2-(4-pyridinyl) - $$ ketone, cyclopropyl4-pyridylmethyl $$ cyclopropyl 4-piclyl ketone $$, Dodecane, 2,7,10-trimethyl-$$ 2,7,10-trimethyl dodecane # $$, Undecane, 2,9-dimethyl- $ $ 2,9-Dimethylundecane # $$, 2-Bromo dodecane $$ Dodecane, 2-Bromo-$$, Undecane,3,9 dimethyl - $$ 3,9-Dimethyl undecane # $$, Ethanone, 1-cyclopropyl 2-(4-pyridinyl) - $$ ketone, cyclopropyl4-pyridylmethyl $$ cyclopropyl 4-piclyl ketone $$ Hexadecane, 1-iodo - $$, Cetyl iodide $$ Hexadecyl iodide $$ 1- iodohexadecane $$ n-Hexadecyl iodide $$ Phenol, 2,6-Bis (1-methylpropyl)- $$ phenol, 2,6-di-sec-butyl-$$ 2,6-Di-sec-butyl fenol $$1-iodoundecane $$ Undecane, 1-iodo- $$, 2-Bromononane $$ sec-Nonyl bromide $$ Nonane, 2-Bromo-$$).

**Table.3. Compounds isolated during somatic embryogenesis on MS basal medium supplemented with 2, 4-D (3.0mg/L) from leaf explants of *Foeniculum vulgare* Mill. using GC/MS**

| Retention Time (min) | Volatile compound isolated during somatic embryogenesis |
|---|---|
| **5.250** | Ethylbenzene (C8H10). Ethylbenzene $$ Benzene, ethyl- $$ Ethylbenzol $$EB$$ Phenylethane $$Aethylbenzol $$ Ethylbenzene $$Etilbenz. Ethylbenzene. Ethylbenzene. o-Xylene (C8H10) |
| **5.550** | o-Xylene. p-Xylene. o-Xylene. Benzene,1,3- dimethyl- . o-Xylene. |
| **6.317** | o-Xylene. o-Xylene. p-Xylene. Benzene,1,3- dimethyl- . p-Xylene. |
| **10.342** | Tridecane, 3-methyl-. Dodecane, 3-methyl-$$ 3-Methyldodecane $$. Undecane, 2,2- dimethyl-. Undecane, 3,9- dimethyl- $$ 3,9- dimethylundecane# $$. |
| **10.542** | 1-Buten-3-yne, 2-tert-butyl-$$ 2-ter-Butyl-1- buten-3-yne # $$,C8H12**.** 5-Hepten-1-yne, 6-methyl-5-heptene-1-yne #$$. Ethanone, 1-cyclopropyl-2-(4-pyridinyl)-$$ Ketone, cyclopropyl 4-pyridylmethyl $$ cyclopropyl 4-picolyl ketone $$1. 1 1,4- Dihydro-2- methylbenzoic acid $$2-Methyl-2,5-cyclohexadiene-1-carboxylic acid #$$(C8H10O2). Ethanone, 1-cyclopropyl-2-(3-pyridinyl)-$$ cyclopropyl 3-picolyl ketone $$ 1- cyclopropyl-2-(3-pyridinyl) ethanone #. (C10H11NO) |
| **12.308** | Nonane, 1-iodo- n$$ n-nonyl iodide $$ 1-Iodononane $$(C9H19I). Dodecane, 1- iodo (C12H25). Octane, 1- iodo(C8H17I). Borane, diethyl(decyloxy)- $$ Decyl diethylborinate #$$(C14H13IBO). Decane,1-iodo |
| **13.942** | Nonane, 3,7-dimethyl-$$ 3,7- Dimethylnonane $$. Dodecane,2,6,11-trimethyl-$$ 2,6,11-Trimethyldodecane $$. Butane, 2,2- dimethyl- , Dodecane, 2,7,10- trimethyl- $$ 2,7,10-trimethyldodecane #$$(C15H32). Undecane, 3, 7-Dimethyl- $$ 3,7-Dimethylundecane #$$(C13H28). |
| **17.375** | Nonane, 3,7-dimethyl-$$ 3,7- Dimethylnonane $$, , 2,7,10- trimethyl- $$ 2,7,10-trimethyldodecane #$$(C15H32). Butane, 2,2- dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3) $$ UN1208 **$$.** |
| **19.250** | Hexane, 3-3- Dimethyl-. Hexane, 3-3- Dimethyl- $$ 3,3 Dimethylhexane $$. Decane, 3, 7-dimethyl-$$ 3, 7-Dimethyldecane #$$. Nonane,1-iodo- $$ n-Nonyl iodide $$ 1-n-Nonyl iodide $$ 1-Iodononane$$. Decane, 4-methyl-$$ 4-Methyldodecane $$. |
| **19.842** | Octane, 2-Bromo-. Octane, 1-Iodo-. 3-Hexane, 2,2- dimethyl-. 1-Iodoundecane $$. Undecane, 1-iodo-$$. 3,5-Dimethyl4-octanone $$. |
| **20.250** | Nonane,1-iodo- $$ n-Nonyl iodide $$ 1-n-Nonyl iodide $$ 1-Iodononane$$. Hexane, 3-3- Dimethyl-. Heptane, 2,5,5-trimethylheptane $$. Octane, 3, 3-dimethyl. 2,3,6,7- tetramethyl-$$ 2,36,7-Tetramethyloctane **#$$** |
| **21.733** | Octane, 2-bromo-. 3-Hexane, 2,2- Dimethyl- . Octane, 1- iodo. 1-Iodoundecane $$ Undecane, 1-iodo-$$. 3,5-Dimethyl-4-octanone $$. |
| **23.033** | Nonane, 1-iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. 2,3,4-Trifluorobenzoic acid,2-tetrahydrofurylmethyl ester $$ Tetrahydro-2-furanlmethyl 2,3,4- |

| | |
|---|---|
| | trifluorobenzoate # $$. Phenol, 4-(1H-pyrol-1-yl)-$$ Phenol,4-pyrol-1-yl)- $$ 4-(1H-pyrol-1-yl) phenol #$$. 2-Bromononane $$ sec-Nonyl bromide $$ Nonane, 2-bromo- $$. Decane, 1-iodo- $$ Decyl iodide $$. 1-Iodododecane $$ 1-Iodododecane$$ n-Decyl iodide $$ 1-Decyl iodide $$. |
| **23.100** | Nonane, 1-iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Dodecane, 1-iodo. Dodecane 4,6 dimethyl-. Dodecane 2,6,11, trimethyl-. Hexane, 3,3- Dimethyl- |
| **23.442** | Pentanedioic acid,(2,4-di-t-butylphenyl) mono ester $$ 5-(2,4-Ditert-butylphenoxy) -5- oxopentanoic acid # $$. Pentanoic acid, Phenol,3,5-bis(1,1-dimethyl- (C10H9NO). Phenol, 2-(1,1-dimethylethyl)-4-(1,1,3,3-tetramethylbutyl)- $$2-tert-Butyl-4-(1,1,3,3-tetramethylbutyl)phenol #$$. |
| **23.775** | Hexane, 3,3- Dimethyl- . Dodecane 2,6,11, trimethyl. Hexane, 3,3- Dimethyl- $$ 3, 3,-Dimethylhexane- $$. 2-Bromododecane $$ Dodecane, 2-bromo-$$. Tetradecane,4- methyl- $$ 4-Methyltetradecane #$$ |
| **25.967** | Nonane, 1-iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Dodecane, 1-iodo. Dodecane 2,6,11, trimethyl-. Dodecane 4,6 dimethyl-, Dodecane 2,6,10, trimethyl |
| **26.500** | Nonane, 1-iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Dodecane 2,6,11, trimethyl-. Dodecane 2,6,10, trimethyl- . 2-Bromo dodecane $$ Dodecane, 2-bromo-$$. Hexane, 3,3- Dimethyl- $$ 3, 3,- Dimethylhexane- $$ |
| **28.400** | Nonane , 1-iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Hexadecane, 1- iodo- $$ Cetyl iodide $$ Hexadecyl iodide $$ 1- Iodohexadecane $$ n-Hexadecyl iodide $$. Tridecanol, 2-ethyl-2-methyl-$$ 2-Ethyl-2-methyl-1-tridecanol #$$. Dodecane, 1-iodo. Tetradecane,4- methyl- $$ 4-Methyltetradecane #$$ |
| **28.858** | Nonane , 1-iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Dodecane, 1-iodo-. Dodecane 2,6,10, trimethyl- . Hexadecane, 1- iodo- $$ Cetyl iodide $$ Hexadecyl iodide $$ 1- Iodohexadecane $$ n-Hexadecyl iodide $$. Dodecane 2,6,11, trimethyl-. |
| **30.567** | Nonane , 1-iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. 2-Bromo dodecane $$ Dodecane,2-bromo-$$. Dodecane, 1-iodo-. Hexadecane, 1- iodo- $$ Cetyl iodide $$ Hexadecyl iodide $$ 1- Iodohexadecane $$ n-Hexadecyl iodide $$. Dodecane 2,6,11, trimethyl- |
| **35.908** | Phenol,2,2'- methylenebis[6-(1,1-dimethylethyl)-4-methyl-$$ p-Cresol, 2,2'-methylenebis[6-tert-butyl-$$ A-22-46$$. Phenol,2,2'- methylenebis[6-(1,1-dimethylethyl)-4-methyl-$$. Phenol, 2,6-bis(1-methylpropyl)- $$ Phenol,2,6-di-sec-butyl-$$ 2,6-Di-sec-butylphenol$$ 2,6-Di-sec-butylfenol$$. Neoisolongifolene-8-ol$$, 1,2-benzenedicarboxylic acid, diisooctyl ester. , Di—n-octylphthalate. 1,2-benzenedicarboxylic acid, diisooctyl ester. Di—n-octylphthalate $$ Hexaplas M/O $$ Isooctyl phthalate $$ Corfl. 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester$$ Mono(2- ethylhexyl)phthalate $$ Phthalic acid, mono-(2-et. Bis(2-ethyhexyl)phthalate $$ 1,2- benzenedicarboxylic acid, bis(2-Ethylhexyl) ester $$ Phthalic acid, bis(2-ethylhexyl). |

**Volatile compounds isolated during regeneration produced on MS Basal medium supplemented with 2, 4- D (3.0 mg/L) from leaf explants of *Foeniculum vulgare* using GC/MS:** One hundred compounds were isolated during regeneration (Table 4 and Fig 2.C) out of which three were novel and named as 2-Bromononane $$ sec-Nonyl bromide $$ Nonane, 2-Bromo-$$, Hexadecane, 1-iodo - $$ Cetyl iodide $$ Hexadecyl iodide $$ 1- iodohexadecane $$ n-Hexadecyl iodide $$ and Phenol, 2,6-Bis (1-methylpropyl)- $$ phenol, 2,6-di-sec-butyl-$$ 2,6-Di-sec-butyl fenol $$).

**Table 4. Volatile compounds isolated during regeneration produced on MS Basal medium supplemented with 2, 4- D (3.0mg/L) from leaf explants of *Foeniculum vulgare* using GC/MS:**

| **Retention Time (min)** | **Volatile compounds isolated during regeneration produced on MS Basal medium supplemented with 2, 4- D (3.0mg/L) from leaf explants of *Foeniculum vulgare* using GC/MS:** |
|---|---|
| **5.267** | Ethylbenzene, Ethylbenzene $$ Benzene, ethyl- $$ Ethylbenzol $$ EB $$ Phenylethane $$Aethylbenzol $$ Ethylbenzene $$ Etilbenz. Ethylbenzene. Ethylbenzene.. N – Benzyloxy-2, 2- (trifluoromethyl)aziridine $$ 1- (Benzyloxy)-2,2-bis(trifluoromethyl)aziridine #$$. |
| **5.558** | o-Xylene. p-Xylene. o-Xylene. p-Xylene. Benzene, 1,3-dimethyl. |
| **6.325** | o-Xylene. p-Xylene. o-Xylene. p-Xylene. Benzene, 1,3-dimethyl. |
| **17.375** | 1-iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. 2-Bromononane $$ sec- Nonyl bromide $$ Nonane, 2-bromo- $$. Nonane, 1-iodo-. Butane, 2,2-dimethyl-. Nonane, 3, 7-dimethyl- $$ 3,7-Dimethylnonane$$. Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3$$ UN 1208 $$. Butane, 2,2-dimethyl- . Butanoic acid, 1,1-Dimethylester $$ CH3CH2CH2C(O)OC(CH3) $$ tert- Butyl butyrate #$$. |
| **19.250** | Nonane, 2- Bromononane $$ sec- Nonyl bromide$$ Nonane,2- Bromo- $$. Nonane, 1-Iodo-. Hexane 3,3-dimethyl. -. Hexane, 3,3- Dimethyl-. 3,5-Dimethyl-4-octane $$. |
| **19.842** | Butane, 2,2-dimethyl- . Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3$$ UN 1208 $$. Butanoic acid, 1,1-Dimethylester $$ CH3CH2CH2C(O)OC(CH3) $$ tert- Butyl butyrate #$$. Butane, 2,2-dimethyl- . Octane, 2-Bromo- |
| **20.250** | 2- Bromononane $$ sec- Nonyl bromide$$ Nonane,2- Bromo- $$. Nonane, 1-Iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Nonane,5-(2—methylpropyl) - $$ 5-Isobutylnonane #$$. Butane, 2,2-dimethyl-. 4- Heptanone, 3-methyl-. |
| **22.700** | Butane, 2,2-dimethyl-. . Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3$$ UN 1208 $$. Butane, 2,2-dimethyl-. 2-Bromo-6-methylheptane$$. Octane, 1-iodo-. |
| **23.042** | Nonane, 1-Iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. 2-Bromononane $$ sec- Nonyl bromide$$ Nonane,2- Bromo- $$. Nonane,5-(2—methylpropyl) - $$ 5-Isobutylnonane #$$. Hexane, 3,3- Dimethyl-. Nonane, 5-methyl-5- propyl-$$ 5- Methyl-5- propylnonane #$$. |

| | |
|---|---|
| **23.775** | Hexane, 3,3- Dimethyl-. Nonane, 1-Iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1-Iodononane $$. 2- Bromononane $$ sec- Nonyl bromide$$ Nonane,2- Bromo- $$. Heptane, 2,5,5-trimethyl-$$ 2,5,5-Trimethylheptane $$.Octane, 3,3- Dimethyl-. |
| **25.967** | Nonane, 1-Iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Octane, 2,3,6,7-tetramethyl-$$ 2,3,6,7-Tetrarthyloctane #$$. Dodecane,1-iodo-. Heptane, 2,5,5-trimethyl-$$ 2,5,5-Trimethylheptane $$. Decane, 3,5,5-trimethyl-$$ 3,3,5-Trimethyldecane #$$. |
| **26.500** | Nonane, 1-Iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Hexane, 3,3-Dimethyl-. Decane, 3,5,5-trimethyl-$$ 3,3,5-Trimethyldecane #$$. Heptane, 2,5,5-trimethyl-$$ 2,5,5-Trimethylheptane $$. Dodecane,1-iodo-. |
| **28.400** | Nonane, 1-Iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Hexane, 3,3-Dimethyl-.$$. Dodecane,1-iodo-. Tetradecane, 4-methyl- $$ 4-Methyltetradecane #$$. 2-Bromo dodecane $$ Dodecane,2- bromo- $$. |
| **28.858** | Nonane, 1-Iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Hexane, 3,3-Dimethyl-.$$. Heptane, 2,5,5-trimethyl-$$ 2,5,5-Trimethylheptane $$. Dodecane,1-iodo-. Decane, 3,5,5-trimethyl-$$ 3,3,5-Trimethyldecane #$$. |
| **30.567** | Nonane, 1-Iodo- $$ n-Nonyl iodide $$ Nonyl iodide $$ 1-n- Nonyl iodide $$ 1- Iodononane $$. Dodecane,1-iodo-. 2-Bromo dodecane $$ Dodecane,2- bromo- $$. Octadecane, 1-Iodo-. 1-Iodoundecane $$ Undecane, 1-iodo- $$. |
| **30.975** | Disulfide, di-tert-dodecyl $$ Di-tert-dodecyl disulfide $$. 1-Hexanol, 5-methyl-2-(1-methylethyl)- $$ 2-Isopropyl- 5-methyl-1-hexanol #$$. 1-Heptanol,5-Methyl-2-(1-methylethyl)- $$ 2-Isopropyl-5- methyl-1-hexanol #$$. 1-Heptanol, 2-propyl- $$ 2-Propylheptanol $$ 2-Propyl- 1-heptanol #$$. Dodecane 2,6,10, trimethyl- . Dodecane 2,6,11, trimethyl-. |
| **33.983** | Phenol,2,2'- methylenebis[6-(1,1-dimethylethyl)-4-methyl-$$ p-Cresol, 2,2'-methylenebis[6-tert-butyl-$$ A-22-46$$. Phenol,2,2'- methylenebis[6-(1,1-dimethylethyl)-4-methyl-. ,2,2'- methylenebis[6-(1,1-dimethylethyl)-4-methyl-. Phenol, 2,6-bis(1-methylpropyl)- $$ Phenol,2,6-di-sec-butyl-$$ 2,6-Di-sec-butylphenol$$ 2,6-Di-sec-butylfenol$$.2,4a-Epidioxy-5,6,7,8-tetrahydro-2,5,5,8a-tetramethyl-2H-1-benzopyran$$. |
| **35.917** | 1,2-benzenedicarboxylic acid, diisooctyl ester. , Di—n-octyl phthalate. 1,2-benzenedicarboxylic acid, diisooctyl ester. , Di—n-octylphthalate $$ Hexaplas M/O $$ Isooctyl phthalate $$ Corfl. 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester$$ Mono(2- ethylhexyl)phthalate $$ Phthalic acid, mono-(2-et. Bis(2-ethyhexyl)phthalate $$ 1,2- benzenedicarboxylic acid, bis92-Ethylhexyl0 ester $$ Phthalic acid, bis(2-ethylhexyl). |

**Discussion**

Leaf explants gave maximum i.e. 81 % callogenesis, 92 % somatic embryogenesis and 90 % regeneration on MS basal medium supplemented with 2, 4-D (3.0 mg/L). Difco bacto agar (0.9 g /L) solidified basal medium with 30 g/L sucrose, under 16 hrs. photoperiod at 23 °C and 5.7 pH was used during whole experiment. Sixty five compounds were identified from callus out of which two were novel. Nagar and Mehwaki (2014) estimated the essential oil composition, total phenolic compounds and total flavonoid compounds in seed and callus induced from two fennel genotypes on MS medium containing different combinations of 2, 4-D and Kinetin. The essential oil description by GC exposed that seed oil had the chief quantity of trans-Anethol of two genotypes.

Parvaneh *et al.* (2014) reported callogenesis and essential oil content changes of hypocotyl explants callus in five fennel populations , Highest Trans-anethole (the main metabolite of fennel) content in fennel callus oil, were observed in Antep population under NAA+BA hormonal treatment.

Similarly, Sharma and Sarin (2012) reported that maximum amount of total sterol was found in 6 weeks old and minimum in 2 weeks old callus cultured *Pedalium murex*. They also isolated stigmasterol present in various plant parts during its tissue cultiurng. During the present study,one hundred and twenty compounds were detected during somatic embryogenesis, out of which nine were found to be novel. Hasanzadeh *et al*. (2004) reported the somatic embryogenesis and isolated essential oil contents in fennel on MS medium under two combined PGRs (NAA + 2,4-D). The highest amount of trans-anethole was observed using GC/MS. Ehsaneh *et al.* (2013) described the production of many essential oils including E, E 2, 4-Decadienal and cineole during callogenesis of Fennel. Saljooghianpour and Javaran (2013) also identified 26 phytochemical compounds including P-Xylene, Tetradecane, Tridecane, Hexadecane and Oleic acid etc. from *in vitro* grown *Aloe vera*. One hundred compounds were identified during regeneration of Fennel in the present study, out of which three found to be novel as mentioned in the results. With reference to the above mentioned work we claim to produce novel bioactive compounds during *in vitro* growth of *Foeniculum vulgare* Mill. which is primarily due to the variations in quality and quantity of the used PGRs during tissue culturing technique. It is also mentionable that quality of the already cited volatile compounds may also be improved during the process and such required volatile compounds of pharmaceutical importance may be obtained for commercial purposes by this green and human friendly method.

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