Micropropagation, Myristicin Production Enhancement, and Comparative GC-MS Analysis of the n-Hexane Extracts of Different Organs of *Daucus pumilus* (Gouan), Family Apiaceae

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**Aim:** This work aimed to study the somatic embryogenesis and organogenesis of endangered *Daucus pumilus* (Gouan) for the conservation of this plant and improving the production of secondary metabolites of medicinal value.

**Materials and Methods:** The callus formation and in vitro propagation of *D. pumilus* (Gouan) by using a different combination of naphthalene acetic acid and benzylaminopurine were established. Various embryogenic stages were tracked using scanning electron microscopy and light microscopy. The volatile constituents of the n-hexane extracts of *D. pumilus* (Gouan) that extracted by ultrasonic-assisted technique were analyzed by gas chromatography–mass spectrometry.

**Results and Discussion:** Somatic embryogenesis and organogenesis of endangered *D. pumilus* (Gouan) were established for the first time. Myristicin and elemicin were successfully increased during micropropagation to 70.89% and 2.19%, respectively. Furthermore, the induction of compounds such as 6-methoxymellein, eugenin, methyl behenate, and 1,6-dimethylnaphthalene was also detected. **Conclusion:** Commercially, this protocol decreases the dependence on wild medicinal plants, enhances the manufacturing of valuable phytochemicals to meet the great demands of the pharmaceutical industries, and acts as a mean for genetic transformation of this plant.

**Keywords** *Daucus pumilus*, gas chromatography–mass spectrometry, myristicin, organogenesis, somatic embryogenesis

INTRODUCTION

Myristicin is the main constituent of *Daucus pumilus* (Gouan) volatile oil,[1] which shows various biological activities, such as hepatoprotective, antibacterial, insecticidal, cytotoxic, anti-inflammatory, and anticholinergic.[2] It was reported that *D. pumilus* (Gouan) is endangered in the Maltese Islands,[3] Provence-Alpes-Côte d’Azur[4] and Jordan.[5] Also, *D. pumilus* (Gouan) is threatened in Egypt[6] and became scarce in Lebanon.[7] In Egypt, the biota of ecosystems has been destroyed in large areas along the coastal regions. This sounds the alarm for the conservation of medicinal plants in the Egyptian coastal dunes.[8] Plant tissue culture techniques enable the repositioning of endangered species and provide a tool for improving the production of metabolites. In addition, these techniques can produce novel compounds that are not naturally synthesized in plants through manipulation of in vitro culture conditions.[9] On the basis of the previous literature, the micropropagation of *D. pumilus* (Gouan) has not been studied. Hence, the first aim of this work was to study the in vitro seed germination,
callus induction, and regeneration of *D. pumilus*. Moreover, investigation of phytochemical production in *D. pumilus* (Gouan) by chemical analysis was the second important goal of this study.

**Materials and Methods**

**Plant material**

The plant material of wild *D. pumilus* (Gouan) (*Pseudorlaya pumila* (L.) Grande) (family: Apiaceae) was collected in March 2014 from the west of Edku, the Beheira Governorate, Egypt. Fruits enclosing seeds were cultivated in the medicinal farm of the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Egypt. The plant was identified by Dr. Hasnna Hosni, Professor of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University, Egypt. A voucher specimen (Accession number of PU-101) was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt.

**Sterilization of seeds**

Wild fruits enclosing seeds of *D. pumilus* (Gouan) were separated into individual mericarps and then sterilized by immersion in 70% ethyl alcohol for 3 min, followed by shaking with 5% sodium hypochlorite solution (Clorox, Okland, CA, USA) containing two drops of 1% tween 20 for 20 min. Under a laminar flow cabinet, the hypochlorite solution was removed, and then the seeds were rinsed three times with sterile distilled water before germination.

**Seed germination in vitro**

Each sterilized mericarp enclosing seeds was cut transversely into two parts and then cultured on sterile Murashige and Skoog (MS) medium (Duchefa Biochemie B.V., Harlem, the Netherlands), supplemented with 30 g/L sucrose (Adwic, Cairo, Egypt) and 8 g/L agar (purified agar for plant tissue culture, BioWorld, Visalia, CA, USA), either alone or in combination with 50 mg/L of gibberelic acid (GA3). The pH was adjusted to 5.8 by 1N HCl or 1N NaOH solution using a Jenway 3510 pH meter before autoclaving at 121°C for 20 min. Four replicates of 26 mericarps enclosing seeds were used for each experiment. All jars were incubated at 25±2°C under a 16 h photoperiod using a white fluorescent lamp. Radicle protrusion of approximately 2 mm was used as a guide for germination of seeds. Seed germination percentage was assessed using the equation reported by Pattanaik et al. \[12\]

**Induction and maintenance of callus**

Five-week-old seedlings were aseptically dissected into small parts (0.5–1 cm) and used as explants for callus formation. Explants were cultivated on sterile MS media containing 3% sucrose, 0.8% agar, and supplemented with various growth regulators (Sigma Chemical, St. Louis, Missouri, USA) combinations as medium I: 0.4 mg/L naphthalene acetic acid (NAA) + 4 mg/L 6-benzylaminopurine (BAP); medium II: 0.1 mg/L NAA+ 1 mg/L BAP; medium III: 1 mg/L NAA+ 0.1 mg/L BAP; medium IV: 2 mg/L 2 mg/L 2, 4 dichlorophenoxy acetic acid (2,4 D) + 1 mg/L kinetin (Kin) and medium V: 0.5 mg/L thiadiazuron (TDZ) + 1 mg/L 2, 4 D + 0.1 mg/L BAP. All media were adjusted to a pH of 5.8, and the cultures were maintained at 25±2°C under 16 h of daily light. Four explants in each jar with four replicates were used for each treatment. Callus cultures were transferred to fresh medium with the same composition every 4 weeks to induce proliferation. The callus induction percentage was measured according to the formula stated by Atabaki et al. \[13\]

**Growth parameters determination**

The growth curve, growth index (GI), specific growth rate, and doubling time (dt) are kinetic tools for evaluating callus growth. Subculturing of induced calli was performed for 2 to 3 generations as a multiplication step before scoring the data. The growth curve was established by plotting the mean value of three replicates of either fresh or dry weight against time. The GI, specific growth rate (μ), and dt were calculated according to equations reported by Godoy-Hernández and Vázquez-Flota. \[14\]

**In vitro propagation of Daucus pumilus (Gouan)**

**Indirect somatic embryogenesis**

After 12–14 weeks on media I, II and III, green embryogenic calli with nodular structures were selected and subcultured on media of the same composition every 28 days for 10 weeks, where development of somatic embryos was monitored by light microscopy. For germination, somatic embryos were transferred to sterile hormone-free MS (HF) medium containing 30 g/L sucrose and 6 g/L agar with a pH of 5.8. Subculturing on fresh media of the same composition was performed twice for 8 weeks.

**Indirect organogenesis**

The non-embryogenic calli on media I and II were carefully subcultured on the same media for 12–16 weeks until the formation of shoot buds. These buds were transferred to medium III or sterile MS solid medium supplemented with 1 mg/L NAA for 8–10 weeks and subcultured every 3 weeks on the same fresh media. All cultures for somatic embryogenesis or organogenesis were maintained at 25±2°C under a white fluorescent lamp with a 16 h photoperiod.
Scanning electron microscopy
Embryogenic calli were prepared for scanning electron microscopy (SEM) according to the general methods for biological sample preparation.[15]

Hardening and acclimatization of the in vitro regenerated plantlets
Two-month-old rooted plantlets were washed with sterile distilled water and out-planted in perforated pots containing autoclaved peat moss soil and sand (1:1). Pots were covered with transparent polyethylene plastic bags to maintain humidity. They were maintained in a growth chamber (25°C and 16 h photoperiod) and irrigated every 2 days with sterile distilled water. After 2 weeks, transparent plastic bags were removed gradually. Finally, pots were transferred to the greenhouse and regularly irrigated to maintain sufficient soil moisture.

Ultrasonic-assisted extraction of volatile constituents
Wild and cultivated D. pumilus (Gouan) fruit, collected leaves and stems, 12-week-old non-organogenic calli (cultivated on media I, II and III), and 8-week-old in vitro regenerated plantlets were extracted separately (each of 25 g) with n-hexane (3 × 150 mL) in a 250-mL volumetric flask and placed in an ultrasonic bath (Sonorex Super RK 103 H, Bandelin, Germany) with a frequency of 35 KHz and a power of 140 W for 15 min at a temperature of 40°C. After ultrasonic-assisted extraction, flasks were kept on a shaker overnight, and the contents were filtered and concentrated by a rotary evaporator (Heidolph rotavapor, Schwabach, Germany) at 45°C and 100 rpm. Hexane extracts were stored in a refrigerator at –4°C until analysis.

Gas chromatography–mass spectrometry analysis
An Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector with a direct capillary interface and fused silica capillary column PAS-5 ms (30 m×0.32 mm ×0.25 µm film thickness) was used for analysis. Samples were dissolved in n-hexane (100 µL/mL), and 1 µL was injected for each sample. Helium was used as the carrier gas at approximately 1 mL/min in pulsed splitless mode, and the solvent delay was 3 min. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from 50 to 500 m/z. The ion source temperature was 230°C. The electron multiplier voltage (EM voltage) was maintained at 1250 V above autotune. The instrument was manually tuned using perfluorotributylamine (PFTBA). The GC temperature program started at 45°C (isothermal for 3 min) and increased to 280°C at 8°C/min, and finally was held isothermally for 10 min. The detector and injector temperatures were set at 280 and 250°C, respectively. The identification of the separated peaks was carried out by matching their mass spectra with those in the literature[16] and mass spectral databases of Wiley and Nist 05. A homologous series of n-alkanes (C8–C24) were injected under similar GC/MS conditions to calculate the retention indices (RI) and were compared with those available in the reported data[16] for further confirmation. The area under the peaks was used to calculate the approximate quantities of the samples using the normalization method.

RESULTS AND DISCUSSION
In vitro seed germination and callus induction
After 5 weeks and 2 days, sterilized dissected seeds were germinated on sterile solid MS medium supplemented with 50 mg/L GA3 only (germination percentage of 12%), as shown in Figure 1A. The low germination percentage in the Apiaceae is due to the presence of seeds lacking embryos (nonviable) or with rudimentary embryos (immature) or dormant embryos.[17] Exogenous application of gibberellic acid (GA3) can break the seed dormancy by allowing oxygen penetration from the outside to the embryo. Furthermore, GA3 has a role in counteracting the inhibitory effect of abscisic acid (ABA). ABA has a main role in the induction of seed dormancy and its maintenance.[18] GA3 can overcome the dormancy of many Apiaceae seeds.[19] Figure 1B shows the seedlings with developed cotyledonary leaves and roots, which reached 3–4 cm in length within 2 weeks and 5 days from germination. After another 2 weeks, the seedlings with true leaves reached 6–7 cm [Figure 1C]. Induction of calli [Figure 1D–F] was only observed when explants excised from 5-week-old seedlings were cultured on media I, II, and III. Pale green friable calli were induced on medium III within 6 weeks. Media I and II produced yellowish-green compact calli after 8 weeks. Media I and III showed the highest callus induction percentage (100%). However, medium II showed only 75% callus induction.

Growth parameter determination
Medium III was the best one for growth, as it showed 6.2- and 11.11-fold increases in callus production in terms of fresh and dry biomass, respectively, during cultivation for 40 days [Figure 2A–C]. Table 1 shows other growth parameters, such as GI, specific growth rate (μ), and dt for all media. Lower dt and higher GI and specific growth rate were observed in calli inoculated on medium III. These previous results confirmed that medium III produced fast-growing calli.
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**Indirect somatic embryogenesis**

Indirect somatic embryogenesis of *D. pumilus* (Gouan) [Figure 3] included formation of pro-embryogenic callus with a globular stage at the induction step within 12 weeks on media I and II and 14 weeks on medium III after a period of callus induction on these media. Notably, a combination of NAA and BAP was efficient at inducing and maintaining somatic embryogenesis. This previous result agrees with those of Shekhawat and Manokari.[20] Embryo development and maturation involved the morphological transitions from globular to heart-shaped to torpedo-shaped, and ultimately cotyledonary stages after another 8 weeks on all media [Figure 3A–D]. Stages of somatic embryogenesis [Figure 3E–I] were monitored by light

### Table 1: Growth parameters of *Daucus pumilus* (Gouan) calli cultivated on media I, II, and III

| Growth parameter       | Growth media |
|------------------------|--------------|
|                        | Medium I     | Medium II   | Medium III  |
| Growth index (GI)      | 4.8*         | 3.9*        | 5.2*        |
| Specific growth rate (μ) | 0.043* g/day | 0.039* g/day | 0.046* g/day |
| Doubling time (dt)     | 16.12 day    | 17.77 day   | 15.06 day   |

*These are the mean of three determinations

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**Figure 1:** Stages of *in vitro* germination of *Daucus pumilus* (Gouan) seeds and callus induction of their seedling explants. (A) Radicle formation after 5 weeks and 2 days. (B) Seedling of 2 weeks and 5 days. (C) Seedling of 5 weeks old. (D, E) Callus induction on media I and II after 8 weeks, respectively. (F) Callus induction on medium III after 6 weeks
microscopy. Within another 2 weeks, all the media showed formation of germinated embryos with elongated radicles [Figure 3J and K]. For the formation of plantlets with well-developed shoots and roots [Figure 3L], germinated embryos were transferred into HF medium for 3 weeks. After another 2 weeks, differentiated plantlets with rootlets [Figure 3M and N] were formed on the same medium. Moreover, umbels of white flowers [Figure 3O] were also formed on the lateral shoots after another 1 week on HF medium. Then, two weeks later, fruits were formed [Figure 3P and Q] on the same medium. Similarly, *in vitro* flower induction on HF medium has been described in many studies and may be related to the endogenous level of auxin. In addition, *in vitro* flowering of *Pimpinella tirupatiensis*, *Dendrocalamus hamiltonii*, and *Brassica nigra* via somatic embryogenesis was achieved. Furthermore, the regeneration of several plants
belonging to the Apiaceae was established through somatic embryogenesis.\[24\]

**Indirect organogenesis**

Organogenic calli of *D. pumilus* (Gouan) were formed with shoot buds on media I and II after 12 and 16 weeks, respectively [Figure 4A and B, respectively]. The proliferation of shoots [Figure 4C] was achieved after the transfer of shoot buds to medium III and medium supplemented with 1 mg/L NAA within 2 and 3 weeks, respectively. The synergistic effect of cytokinin (BAP) in combination with auxin (NAA) in medium III led to an increase in lateral shoot number and elongation compared to that achieved with medium supplemented with NAA only. This finding is in agreement with that reported by Palengara.\[25\] Moreover, rooting was established on either medium supplemented with
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1 mg/L NAA [Figure 4D] or medium III [Figure 4E] within 4 weeks after transfer. The use of 1 mg/L NAA for root formation is consistent with methods reported for *D. carota* L.[26] While rooting of *D. carota* subsp. *halophilus* shoots was established on medium without plant growth regulators.[24] Moreover, medium supplemented with 1 mg/L NAA resulted in the formation of a flower cluster [Figure 4F] of 2–3 flowers within 5 weeks. After 8 weeks on medium III, a cluster of flowers (inflorescence) [Figure 4G] on the main stem and other clusters of 2–3 flowers on the lateral stems were observed. Similarly, *in vitro* flowering during organogenesis occurred in some plants belonging to Apiaceae.[24] Furthermore, fruit formation was observed after 8 and 10 weeks on medium supplemented with 1 mg/L NAA [Figure 4H] and medium III [Figure 4I],
respectively. In addition, in vitro fruiting of *Ammi majus* L.[27] via direct organogenesis was the only previous example in Apiaceae. However, many plants belonging to Apiaceae have been regenerated through organogenesis.[24]

**Scanning electron microscopy**
SEM showed the presence of numerous globular and heart-shaped forms [Figure 5A and B, respectively]. On the contrary, late heart-shaped [Figure 5C and D], torpedo-shaped, and cotyledonary forms [Figure 5E and F, respectively] were rarely found. Furthermore, this study revealed the efficiency of the combination of NAA and BAP for the induction, development, and maturation of embryogenic calli.

**Hardening and acclimatization of the in vitro regenerated plantlets**
Only 4% of the plants [Figure 6A-D] survived for 6 weeks after transfer to the greenhouse. Moreover, two-month-old rooted plantlets with fruits [Figure 6E and F] survived for only 2 weeks after transplantation. Similarly, a low survival rate of plants belonging to Apiaceae was reported previously.[28] Moreover, the difficulty in the acclimatization of Apiaceae plants has been attributed to fungal rots.[29]

**Gas chromatography–mass spectrometry analysis**
The gas chromatography–mass spectrometry analysis (GC-MS) analysis of the volatile constituents of the *n*-hexane extracts of the in vitro regenerated plantlets and different organs of wild and cultivated *D. pumilus* (Gouan) is shown in Supplementary Table 1 [Additional file 1]. A total of 39, 44 and 16 compounds were identified for different organs of wild and cultivated *D. pumilus* (Gouan) and in vitro regenerated plantlets, respectively. The results showed the presence of 23 similar compounds in both wild and cultivated *D. pumilus* (Gouan). Myristicin was the major constituent of fruits and collected leaves and stems of wild and cultivated *D. pumilus* (Gouan) at different concentrations (46.12%, 27.66%, 26.98%, and 2.51%, respectively). This difference between wild and cultivated plants may be due to geographic and climatic factors.[30] A noteworthy feature of this study is that the phenylpropanoids; myristicin and elemicin were successfully increased during the micropropagation method to 70.89% and 2.19% in

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*Figure 5: Scanning electron microscopy (SEM) of different stages of somatic embryos in Daucus pumilus (Gouan), including globular (A), heart-shaped (B), late heart-shaped (C, D), torpedo (E), and cotyledonary (F)*
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The *in vitro* regenerated plantlets, respectively. Similar results were reported for *Ocimum basilicum* plantlets in which the combination of auxin and BAP led to the synthesis of phenylpropanoids (estragole). The induction of compounds such as 6-methoxymellein (0.42%), eugenin (1.31%), methyl behenate (0.48%), and 1,6-dimethylnaphthalene (0.14%) in the *in vitro* regenerated plantlets of *D. pumilus* (Gouan) was detected. Furthermore, this study revealed the absence of any volatile constituents in the *n*-hexane extracts of all calli except for the production of 6-methoxymellein in a percentage of 11.35% and 17.45% in calli growing on media I and II, respectively. Moreover, 7.78% eugenin was produced only in calli cultivated on medium II. Similarly, 6-methoxymellein and eugenin were produced in cultured *D. carota* L. and in roots of field-grown plants of the same species in response to stresses such as ethylene, methyl jasmonate, 2,4-D, UV-B irradiation and fungal inoculation. Furthermore, new volatile constituents were produced in plantlets of *Eucalyptus camaldulensis* Dehnh. that regenerated from a nodal explant using a combination of NAA and BAP. In addition, carvone was absent in *Mentha spicata* L. callus, whereas it was produced in the cultured plantlets. In addition, many researchers stated that the biosynthesis of secondary metabolites is dependent on cell differentiation and restricted to specific organs, which explains why myristicin and other volatile constituents in this study were produced in the *in vitro* regenerated plantlets but not produced in callus.

Regeneration of *D. pumilus* (Gouan) *in vitro* was successfully established via indirect somatic embryogenesis and organogenesis for the first time. *In vitro* flowering and fruiting of *D. pumilus* (Gouan) are an amazing mean for completing the life cycle of this plant. Consequently, the biosynthetic machinery necessary to produce metabolites can be achieved inside jars without the need for *ex vitro* acclimatization. GC-MS revealed that myristicin and elemicin were successfully elevated and new compounds were induced in the *n*-hexane extract of the *in vitro* regenerated plantlets. Therefore, this result highlights that *in vitro* cell cultures of *D. pumilus* (Gouan) can act as biofactories.

**Figure 6: Hardening and acclimatization of the *in vitro* regenerated *Daucus pumilus* (Gouan) plantlets.** (A, B) Two-month-old rooted plantlets, (C) plantlets after 2 weeks of transplantation, plantlets started to wilt after 6 weeks (D) or after 10 days (E) of transplantation, (F) 2.5-month-old rooted plantlets with fruits.
for secondary metabolite production, independent of the availability of wild plants or appropriate climatic conditions. Commercially, this protocol can be used as a tool for the conservation strategy of *D. pumilus* (Gouan) and to enhance the manufacturing of valuable phytochemicals to meet the great demands of the pharmaceutical industries. In addition, these methods decrease the dependence on wild medicinal plants and acting as a mean for genetic transformation of this plant.

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**Conflicts of interest**

There are no conflicts of interest.

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### Supplementary Table 1: The volatile constituents of the *n*-hexane extracts of the *in vitro* regenerated plantlets and different organs of wild and cultivated *D. pumilus* (Gouan)

| No. | Name                        | Calculated RI | Reported RI | M⁺ (m/z) | Base peak (m/z) | RP % | WP % | CP % | F L & S | F L & S |
|-----|-----------------------------|----------------|-------------|----------|-----------------|------|------|------|---------|---------|
| 1   | Limonene                    | 1032           | 1029        | 136      | 68              | -    | 0.17 | -    | -       | -       |
| 2   | *trans*-Decahydronaphthalene| 1059           | 1059        | 138      | 138             | -    | 0.12 | -    | -       | -       |
| 3   | *n*-Undecane                | 1104           | 1100        | 156      | 57              | -    | 2.41*| 0.25*| 1.97*   | -       |
| 4   | 2-Methylundecane            | 1167           | ***         | 170      | 57              | -    | -    | -    | 4.01    | -       |
| 5   | *n*-Dodecane                | 1201           | 1200        | 170      | 57              | 5.51*| 7.54*| 17.62*| 2.54*   | -       |
| 6   | 2,6-Dimethylundecane        | 1215           | ***         | 184      | 57              | -    | 1.25*| 3.84*| 0.56*   | -       |
| 7   | Hexylcyclohexane            | 1242           | ***         | 168      | 83              | -    | 0.50*| 0.97*| 0.06*   | -       |
| 8   | 2-Methylundecane            | 1267           | ***         | 184      | 57              | -    | -    | 1.03  | -       | -       |
| 9   | *cis*-Chrysanthenyl acetate | 1267           | 1265        | 192      | 192             | 70.89| 46.12*| 27.66*| 26.98*  | 2.51*   |
| 10  | *cis*-Verbenyl acetate      | 1270           | 1282        | 194      | 119             | 4.49 | -    | -    | -       | -       |
| 11  | Bornyl acetate              | 1294           | 1288        | 196      | 95              | -    | 0.55 | 0.22 | -       | -       |
| 12  | *n*-Tridecane               | 1304           | 1300        | 184      | 57              | -    | 0.26*| 0.48*| 0.86*   | 0.38*   |
| 13  | 2-Methyltridecane           | 1366           | ***         | 198      | 57              | -    | -    | 0.39  | -       | -       |
| 14  | *α*-Cubebene                | 1385           | 1348        | 204      | 161             | -    | 0.37 | -    | -       | -       |
| 15  | *n*-Tetradecane             | 1400           | 1400        | 198      | 57              | 0.25 | 0.31*| 1.13*| 1.42*   | 1.02*   |
| 16  | 1,6-Dimethylnapthalene      | 1412           | ***         | 156      | 156             | 0.14 | -    | -    | -       | -       |
| 17  | 2,6-Dimethylnapthalene      | 1412           | ***         | 156      | 156             | 0.31 | -    | 0.58 | 0.38    | -       |
| 18  | 2,7-Dimethylnapthalene      | 1428           | ***         | 156      | 156             | 0.31 | -    | 0.13*| 0.17    | -       |
| 19  | *β*-Caryophyllene           | 1431           | 1419        | 204      | 133             | 0.59 | -    | -    | -       | -       |
| 20  | *α*-Humulene                | 1469           | 1454        | 204      | 93              | 5.99*| 1.80*| 0.92*| -       | -       |
| 21  | Germacrene-D                | 1495           | 1485        | 204      | 161             | 0.12 | -    | -    | -       | -       |
| 22  | *n*-Pentadecane             | 1503           | 1500        | 212      | 57              | 0.38 | 0.29*| 1.27*| 1.47*   | 1.44*   |
| 23  | *β*-Bisabolene              | 1519           | 1505        | 204      | 93              | 3.45*| 1.41*| 0.81*| -       | -       |
| 24  | Myristicin                  | 1532           | 1518        | 192      | 192             | 70.89| 46.12*| 27.66*| 26.98*  | 2.51*   |
| 25  | 2,3,6-Trimethylnapthalene   | 1557           | ***         | 170      | 170             | -    | -    | -    | 0.19    | -       |
| 26  | Elemicin                    | 1561           | 1557        | 208      | 208             | 2.19 | 1.98*| 0.70*| 0.77*   | -       |
| 27  | *n*-Hexadecane              | 1600           | 1600        | 226      | 57              | 0.44 | 0.59*| 1.37*| 1.34*   | 1.47*   |
| 28  | Humulene epoxide II         | 1629           | 1608        | 220      | 109             | 1.26 | 2.41 | -    | -       | -       |
| 29  | *α*-Bisabolol               | 1698           | 1685        | 222      | 109             | 0.22 | 3.22*| -    | 0.43*   | -       |
| 30  | *n*-Heptadecane             | 1703           | 1700        | 240      | 57              | 0.41 | -    | 0.91 | 1.30    | -       |
| 31  | Shyobunol                   | 1710           | 1689        | 222      | 84              | 0.92 | -    | -    | -       | -       |
| 32  | Pristane                    | 1710           | ***         | 268      | 57              | -    | -    | -    | 0.52    | -       |
| 33  | Undecylbenzene              | 1785           | ***         | 232      | 92              | -    | 0.31 | -    | -       | -       |
| 34  | *n*-Octadecane              | 1801           | 1800        | 254      | 57              | -    | -    | 0.82 | 1       | -       |
| 35  | Hexahydrofarnesyl acetone   | 1850           | ***         | 268      | 58              | -    | -    | 0.35 | 0.47    | -       |
| 36  | 6-Methoxymellein            | 1858           | ***         | 208      | 164             | 0.42 | -    | -    | -       | -       |
| 37  | *n*-Nonadecane              | 1903           | 1900        | 268      | 57              | -    | 0.69*| 0.62*| 1.13*   | -       |
| 38  | *n*-Heneicosane             | 2106           | 2100        | 296      | 57              | -    | -    | -    | -       | -       |
| 39  | *n*-Tetracosane             | 2257           | ***         | 262      | 92              | -    | 0.55 | -    | 0.89    | -       |
| 40  | *n*-Pentacosane             | 2257           | ***         | 262      | 92              | -    | 0.55 | -    | 0.89    | -       |
| 41  | Methyl behenate             | 2336           | ***         | 326      | 74              | 0.48 | -    | -    | -       | -       |
| 42  | *n*-Tetracosane             | 2401           | 2400        | 338      | 57              | -    | 0.34*| 0.35*| 0.68*   | -       |
| 43  | *n*-Pentacosane             | 2506           | 2500        | 352      | 57              | -    | 0.16*| 0.55*| 0.89*   | -       |
| 44  | Methyl heptadecane          | 2538           | ***         | 354      | 74              | 0.48 | -    | -    | -       | -       |
### Supplementary Table 1: Continued

| No. | Name                          | Calculated RI | Reported RI | M⁺ (m/z) | Base peak (m/z) | RP % | WP % | CP % |
|-----|-------------------------------|---------------|-------------|----------|-----------------|------|------|------|
| 55  | n-Heptacosane                 | 2705          | 2700        | 380      | 57              | -    | 2.36*| -    |
| 56  | Visnadine                     | 2724          | ***         | 388      | 229             | -    | -    | 1.83 |
| 57  | Methyl lignocerate            | 2737          | ***         | 382      | 74              | 1.74 | -    | 0.40 |
| 58  | n-Octacosane                  | 2801          | 2800        | 394      | 57              | -    | 0.19 | -    |
| 59  | Squalene                      | 2837          | ***         | 410      | 69              | -    | 0.20 | -    |
| 60  | n-Nonacosane                  | 2908          | 2900        | 408      | 57              | -    | 4.15*| 4.03*| 7.10*|
| 61  | Campesterol                   | 3441          | ***         | 400      | 400             | -    | 0.30 | -    |
| 62  | Stigmasterol                  | 3511          | ***         | 412      | 412             | -    | 1.30*| 4.73*| 1.65*| 23.69*|
| 63  | β-Sitosterol                  | 3645          | ***         | 414      | 414             | -    | 1.56*| 5.01*| 3.28*| 7.27* |
| 64  | β-Amyrin                      | 3725          | ***         | 426      | 218             | -    | 0.74 | -    |
| 65  | 5α-Stigmast-7-en-3β-ol        | 3781          | ***         | 414      | 414             | -    | 0.36*| 1.57*| 0.55*|

#### Reported RI
- Monoterpene hydrocarbons: 0.17
- Monoterpene esters: 5.04
- Sesquiterpene hydrocarbons: 10.15
- Oxygenated sesquiterpenes: 0.22
- Diterpene alcohols: 0.03
- Triterpene hydrocarbons: 0.20
- Pentacyclic triterpenes: 0.74
- Phenylpropanoids: 73.08
- Isoprenoid alkanes: -
- Coumarin derivatives: 0.42
- Chromone derivatives: 1.31
- Phytosterols: -
- Higher alkanes: 2.31
- Others: 2.67
- Total identified compounds: 80.04

Calculated RI: Retention indices calculated in relative to C8–C24 n-alkanes on the fused silica capillary column PAS-5ms, Reported RI: Reported retention indices,\(^{[18]}\) RP: In vitro regenerated plantlets, WP: Wild plant, CP: Cultivated plant, F: Fruit, L & S: Leaf & stem, *: Similar compounds between wild and cultivated plants, **: Identified by comparing mass fragmentation with that found in the literature,\(^{[32,37]}\)**: Identified by comparing mass spectra and Kovats retention indices with NIST Standard Reference Data (https://www.nist.gov/srd), ****: Identified by comparing mass fragmentation with that found in the literature.\(^{[32]}\)