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Integrated GC-MS and UPLC-ESI-QTOF-MS based untargeted metabolomics analysis of *in vitro* raised tissues of *Digitalis purpurea* L

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Digitalis purpurea L. is one of the important plant species of Nilgiris, Kashmir and Darjeeling regions of India, belonging to the family Plantaginaceae, with well-known pharmacological applications. In the present investigation, an *in vitro* culture technique of indirect shoot organogenesis of *D. purpurea* is being explored; the biochemical attributes, the antioxidant activities and the metabolomic analyses were made by utilizing untargeted Gas Chromatography-Mass Spectrometry (GC-MS) and Ultra Performance Liquid Chromatography coupled with electronspray ionization/quadrupole-time-of-flight-mass spectrometry (UPLC-ESI-QTOF-MS) approaches. Initially, the leaf explants were used for callus induction and proliferation and maximum callusing frequency (94.44%) and fresh biomass (4.9 g) were obtained on MS, fortified with 8.8 µM BAP (6-benzyl amino purine) + 0.9 µM 2,4-D (2,4-dichlorophenoxyacetic acid), subsequently shoot formation (indirect organogenesis) was noted on the same MS medium with a shoot induction frequency of 83.33%. Later on, the biochemical and antioxidant potential of *in vivo*-, *in vitro* grown leaf and leaf derived callus were assessed. Significantly higher total phenol, flavonoid, DPPH (2,2-diphenyl-1-picrylhydrazyl), POD (peroxidase) and SOD (superoxide dismutase) activities were noticed in *in vitro* grown callus and leaf tissues compared with field grown leaf. The GC-MS analysis of each methanolic extract (*in vivo*-, *in vitro* derived leaf and leaf derived callus) displayed the presence of more than 75 bioactive compounds viz loliolide, stigmasterin, alpha-tocopherol, squalene, palmitic acid, linoleic acid, beta-amyrin, campesterol etc. possessing immense therapeutic importance. The UPLC-MS based metabolite fingerprinting of each methanolic extracts were conducted in both positive and negative ionization mode. The obtained results revealed variation in phytochemical composition in field - and laboratory grown tissues, indicating the impact of *in vitro* culture conditions on plant tissues. The detected phytocompounds belongs to various classes such as flavonoids, steroids, terpenoids, carbohydrates, tannins, lignans etc. The medicinally important metabolites identified were 20, 22-dihydrodigoxigenin, digoxigenin monodigitoxoside, apigenin, luteolin, kaempferide, rosmarinic acid, nepitrin

and others. The results of the present study suggest that *in vitro* culture of *D. purpurea* could successfully be utilized for the novel drug discovery by producing such important phytocompounds of commercial interest in shorter duration without harming the plants' natural population.

KEYWORDS

metabolomics, indirect organogenesis, biochemical, SOD, POD, GC-MS, UPLC-ESI-QTOF-MS, cardenolides

1 Introduction

For decades, medicinal plants have been a crucial source for pharmaceutical sector as more than half of the world's populations are still reliant on traditional medicine system (Gomathi et al., 2013). Synthetically designed chemical drugs are often associated with multiple side effects, whereas plant-based medicines are derived naturally and thus, are more sustainable with negligible side effects (Konappa et al., 2020). Medicinal plants are frequently used in traditional medicine, food, cosmetics and healthcare as the plants are enriched with diverse groups of bioactive compounds which can be extracted for industrial and commercial purposes (Shakya, 2016; Rad et al., 2021). Plants produce a vast array of therapeutically important secondary metabolites like alkaloids, flavonoids, lignans, terpenoids, steroids and anthocyanins, along with significant primary metabolites like lipids, sugars and amino acids (Mickymaray, 2019; Thakur et al., 2019) play not only a decisive role in plant growth, development and reproductive cycle but also in defensive mechanisms (Singh et al., 2020). Several bioactive compounds exhibit crucial biological activities such as antineoplastic, anti-proliferative, anti-aging, anti-inflammatory, anti-angiogenic, anti-microbial, antiviral properties etc (Altemimi et al., 2017; Anand et al., 2019).

Digitalis purpurea L. (Plantaginaceae family) is an important biannual herbaceous plant with both ornamental and medicinal values and is commonly known as foxglove (Al-Oqab et al., 2022). The plant is indigenous to Europe and is grown in the Nilgiris, Kashmir and Darjeeling regions (Rehman Nengroo and Rauf, 2020). The well-known feature of *D. purpurea* is its tall spike-borne, campanulate flowers with color ranging from purple, pink, yellow, or white (Wu et al., 2012). It is currently a popular source of characteristic bioactive compounds cardenolides (digoxin, digitoxin, gitaloxin, gitoxin, strospeside); flavonoids (digicitrin and cyaniding); anthraquinone (digiferruginol), phenylethanoids (cornoside and maxoside) etc (Kreis, 2017; Amiri et al., 2023). These plant-based compounds exhibit multiple impact on health such as neuro-, hepato- and cardioprotective, anti-diabetic, anti-viral, anti-cancerous and cytotoxic activities (Verma et al., 2016a; Nartop et al., 2021). Digoxin and digitoxin are significant cardiotonic glycosides that are used to treat atrial arrhythmia and congestive heart failure (CHF) (Bhusare et al., 2018), by modifying the heart muscles' contractile force and aid in their inotropic actions (Patel, 2016).

The evaluation of such phytocompounds as potent novel drugs requires simple efficient extraction, compound identification, economical production, employment of host organisms, conduction of preclinical, clinical trials and quantitative structure-activity relationship (QSAR) studies and complete metabolomic profiling of source plants (Dey et al., 2020). However, several factors restricting commercial production of these phytocompounds from plants growing in the wild include poor accessibility, over-utilization, cultivation challenges, low production, seasonal fluctuations, complications in extraction, extent of impurities, and the financial cost associated with suitable screening biological assays (Halder et al., 2019). In such scenario, micropropagation techniques prove to be beneficial in producing elite clones that synthesize adequate amount of phytocompounds in a shorter period of time without harming natural habitat (Ajithan et al., 2019; Erişen et al., 2020). *In vitro* regenerated plantlets can be produced by two fascinating biotechnological methods: somatic embryogenesis and organogenesis (Bansal et al., 2022). The *in vitro* production of bioactive compounds can be obtained from callus, cell suspension, shootlets, roots etc. and the whole process consists of two steps: (i) biomass aggregation and (ii) phytocompounds biosynthesis and extraction (Chandran et al., 2020).

After transcriptomics and proteomics, the study of metabolites-related events in living organisms, or metabolomics has emerged as the third significant area of functional genomics (Deepalakshmi et al., 2016). Thus, the integration of *in vitro* plant cultures with metabolomics investigations opens significant avenues to analyze the bioactive profile of different plant samples qualitatively and quantitatively (Carla Guimarães Sobrinho et al., 2022). Furthermore, metabolomic information will offer exceptional perspectives on underlying characteristics of plant phenotypes concerning development, physiology, tissue identity, resistance, biodiversity, and so forth (Hall et al., 2002). A wide range of analytical techniques including Gas Chromatography-Mass Spectrometry (GC–MS), Nuclear Magnetic Resonance (NMR) (Abdelsalam et al., 2017), Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC–MS) (Oliveira et al., 2018), Capillary Electrophoresis (CE) and High-Performance Liquid Chromatography-Mass Spectrometry (HPLC–MS)

(Marchetti et al., 2019) have been employed for high-throughput analysis of metabolites over the past ten years.

Because of the extremely diverse biochemistry of plants, and presence of several important bioactive groups, both the GC-MS and LC-MS techniques are most frequently used in detecting critical phytocompounds (El Sayed et al., 2020). In metabolomics analysis, the MS is used in conjunction with GC or LC to leverage the advantages of each technique-the robustness of MS detectors and the higher resolution and reproducibility of chromatographic system. The GC-MS is often used for the study of volatile organic compounds, lipids and derivatizable compounds, whereas LC-MS is typically used for the investigation of mostly semi-polar metabolites (Zeki et al., 2020). These techniques have recently been applied to different *in vitro* raised plant tissues like *Leucojum aestivum, Saraca asoca* and *Pluchea lanceolata* (Spina et al., 2021; Mamgain et al., 2022; Vignesh et al., 2022).

Considering all these aspects, the information regarding the metabolic profiling of *in vitro* raised tissues of *D. purpurea* is still unknown, so it would provide insights about the plants' phytochemistry and pharmacological importance. Thus, in the present work, we report the comparative and untargeted metabolite profiling of leaf-callus and *in vivo*- and *in vitro*-raised leaf tissues of *D. purpurea* by GC-MS and LC-MS techniques. The biochemical attributes such as total phenolic content, total flavonoid content and anti-oxidant activities were also assessed in cultured tissues during metabolite accumulation in *D. purpurea*. This is the first report of its kind which may auger novel new drug development in future.

2 Materials and methods

2.1 Chemicals and reagents

All the chemicals and solvents used in this study were of analytical grade. MS medium (Murashige and Skoog, 1962), methanol, Folin-ciocalteu reagent, Gallic acid, Quercetin, 2,2-Diphenyl-1-picrylhydrazy (DPPH), hydrogen peroxide, Triton X-100, ethylenediaminetetraacetic acid (EDTA), polyvinylpyrollidone (PVP), methionine, Nitro blue tetrazolium (NBT), riboflavin and all other chemicals and reagents were purchased from Himedia (Mumbai, India), SRL (Mumbai, India) and Sigma Aldrich (USA).

2.2 Explant preparation and *in vitro* culture conditions

Healthy and young leaves of *D. purpurea* were collected and underwent surface sterilization following the protocol of Bansal et al. (2022). Under sterilized conditions of laminar air flow, the leaf explants (3-4 cm) were inoculated on MS medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. After adjusting the pH to 5.8, the medium was sterilized for 15 min at 121°C at 1.06 kg/cm^2 of pressure. The culture was maintained at 24 ± 2°C in the dark for initial two days, and then switched to a 16:8h photoperiod cycle (cool white fluorescent light with an intensity of 50 µmol/m^2/s^{-1}).

2.3 Callus induction and indirect organogenesis

For callus induction, the leaf explants of *D. purpurea* were cultured onto MS medium supplemented with a combination of 6-benzylaminopurine (BAP) and 2,4-dichlorophenoxy-acetic acid (2,4-D) at varying concentrations. After 4 weeks, the produced callus was sub-cultured on the same medium for another 4 weeks to obtain shoots (indirect organogenesis). Each treatment included five replicates (one explant/test tube), and every experiment was conducted thrice. The callus induction percentage (%), fresh biomass (g) as well as indirect shoot induction percentage (%) and the mean shoot numbers/callus mass were recorded after 4 weeks period.

2.4 Biochemical analyses

2.4.1 Sample preparation

The leaf derived calli and *in vivo* and *in vitro* (organogenic derived) grown leaf tissue of *D. purpurea* were collected and air dried at room temperature for 5 days. Using a mortar and pestle, around 1.0 g of each air-dried sample was crushed into a fine powder. Each sample was then separately extracted using 10 mL of methanol (MeOH) solvent on a rotary shaker for 2 days, followed by filtration of extracts with Whatman filter paper No. 1. The filtered samples were then centrifuged for five min at 12,000 rpm, and the recovered supernatant was stored at 4°C for further use.

2.4.2 Total phenolic content estimation

The TPC determination was carried out with Folin-Ciocalteu protocol (Baliyan et al., 2022). Firstly, 2.5 mL of 10% (v/v) Folin-Ciocalteu (FC) reagent ((Sigma-Aldrich, New York, NY, USA) was thoroughly mixed with approximately 0.5 mL of extract and kept at room temperature (RT) for 5 min. After that, 2 mL of 7% Na_2C0_3 was added, followed by incubation for 90 min at RT. Then, the absorbance of each sample was measured at a wavelength of 765 nm by using a UV-Vis spectrophotometer (Biolinkk, BL-295, Delhi, India) against a blank. The experiment was conducted in triplicates and a calibration curve of gallic acid was prepared to determine the total phenolic content in each sample. Results were expressed as milligrams of Gallic acid equivalents per gram of Dry Weight (mg GAE/g DW).

2.4.3 Total flavonoid content estimation

The TFC determination was conducted following the protocol reported by Aryal et al. (2019). The first step involved mixing 1.0 mL of extract (sample) solutions with 0.2 mL of 10% $AlCl_3$ and 0.2 mL of 1 M potassium acetate solution. Later, 3.6 mL of distilled water was

added to the total reaction volume, which was then allowed to incubate at room temperature for 30 min. After fully mixing the aforementioned solution, the absorbance at 510 nm was measured using a UV-visible spectrophotometer against a blank. Every TFC determination was carried out in triplicate and a calibration curve of quercetin was plotted to determine the total flavonoid content in each sample. Results were expressed as milligrams of Quercetin Equivalents per gram of Dry Weight (mg QE/g DW).

2.4.4 DPPH radical scavenging activity assay

The free radical scavenging activity (FRSA) of extract samples of *D. purpurea* was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Baliyan et al. (2022) protocol. Approximately 100 µL of methanolic extracts (samples) were taken in separate test tubes and mixed with 3.0 mL DPPH (0.024% w/v). As a reference standard, 100 µL of methanol was mixed with 3.0 mL of DPPH. The samples were then incubated at room temperature for 90 min in complete darkness and the absorbance was checked at 517 nm wavelength. The following equation was used to determine the anti-oxidant potential of each sample (Khan H. et al., 2021):

$$\% \text{ Scavenging} = (A_C - A_S/A_C) \times 100$$

where A_C = recorded absorbance of control and A_S = recorded absorbance of sample.

2.4.5 Determination of peroxidase (POD; EC: 1.11.1.7) activity

The sample preparation and POD assay were carried out in accordance with Bansal et al.'s (2024) procedure. 10 mL of 0.1M phosphate buffer (pH=6.0) was taken to homogenize 1.0g of fresh samples (*in vivo*-, *in vitro*-raised leaf tissues and leaf derived callus). The extracts were then filtered, centrifuged at 12000 rpm for about 30 min at 4°C and the supernatants were collected. The samples were preheated at 65°C for 50 secs and then refrigerated until needed. A reaction mixture containing 1.0 mL of 10 mM K-phosphate buffer (pH = 7.0), 500µL of 1% guaiacol solution, 500µL hydrogen peroxide solution (0.4%), 500µL of enzyme extract, and 2.5 mL of distilled water was used for the peroxidase enzyme assay. The control was prepared with all the above stated reagents, except the enzyme extract. The development of tetraguaiacol was then confirmed by measuring the increase in absorbance at 470 nm within 30 min. The following formula was used to determine the enzymatic activity:

$$A = ELC$$

Wherein A = absorbance, E = extinction coefficient (6.39 $mM^{-1}cm^{-1}$), L = path length (1.0 cm) and C = enzyme concentration (mM/g FW), and FW = fresh weight of samples.

2.4.6 Determination of superoxide dismutase (SOD; EC: 1.15.1.1) activity

Following the protocol provided by Mujib et al. (2022), the enzyme extract preparation and SOD assay were performed. Initially, 1.0 g of all the three fresh tissue samples were homogenized in 10 mL of 0.5 M sodium phosphate buffer (pH 7.3), containing 1.0% (v/v) Triton X-100, 3.0 mM ethylenediaminetetraacetic acid (EDTA), and 1.0% (w/v) polyvinylpyrollidone (PVP), to prepare the enzyme extracts. Finally, the supernatant was collected after the homogenate was filtered and centrifuged for 15 min at 4°C at 11,800 x g.

The SOD assay was conducted using a final reaction mixture of 3.0 mL, consisting of 50 mM K-phosphate buffer (pH 7.8), 45 µM methionine, 1.0 M Na_2CO_3, 2.25 mM Nitro blue tetrazolium (NBT) solution, 3.0 mM EDTA, 10 µM riboflavin, 10 µL of enzyme extract, and distilled water. A control group was established in which no enzyme extract was included. Subsequently, the mixture was subject to incubation at a temperature of 25°C for a duration of 10 min, while being exposed to fluorescent lamps with a power output of 15 W. The spectrophotometer was used to measure the absorbance of each sample at a wavelength of 560 nm. One unit of superoxide dismutase (SOD) activity is defined as the amount of enzyme required for 50% inhibition of NBT reduction. The activity is measured in units (U) per milligram of fresh weight (mg FW).

2.5 Metabolomics study using untargeted GC-MS approach

2.5.1 Preparation of extracts

For GC-MS analyses, approximately 1 g of each air-dried leaf tissue (*in vivo*- and *in vitro* grown) and air-dried leaf derived callus was finely grounded with a mortar and pestle. Each powdered sample was then individually macerated with MeOH at room temperature for 48h on an orbital shaker. The resulting extracts were filtered using Whatman No. 1 filter paper and centrifuged at 10,000 rpm for 5 min. Later, the supernatants were filtered using a syringe filter (0.22 µm, Genetix, New Delhi, India) and stored at 4°C for metabolite analyses.

2.5.2 GC-MS instrumentation and data analyses

The methanolic extracts of the samples were analyzed by GC-MS-QP-2010 equipment (Shimadzu, Tokyo, Japan), with the following parameters: The GC-MS separation was performed using the Rxi-5Sil MS GC Capillary Column (30 m, 0.25 mm ID, 0.25 µm film thickness). Helium was employed as the carrier gas at a consistent flow rate of 1.21 mL min−1. A GC-MS detection method utilized an electron impact ionization mode with ionization energy of 70 eV. The inlet temperature was set to 260°C, initial oven temperature was adjusted to 80°C and was programmed to increase to 280°C (hold time of 18 min) with a sample injection volume of 1 µl and scanning range of 40-600 m/z. For GC-MS analysis, the identification of phytocompounds present in each sample was done by comparing their retention times, peak area and peak area % to those of authenticated compounds listed in the database of NIST (National Institute of Standards and Technology) using GCMS solution software (Version 4.45 SP 1).

2.6 UPLC-ESI-QTOF-MS based metabolites profiling

2.6.1 Sample preparation and metabolite extraction

The samples were prepared by shade drying each plant material (*in vivo*-, *in vitro* grown and leaf derived calli) of *D. purpurea* for 10-12 days. The shade dried materials were then finely grounded into powder and 50 mg per sample was utilized for metabolites extraction for LC-MS analysis. The powdered samples were extracted with 1.0 mL methanol (MeOH), sonicated at 40 kHz for 15 min at room temperature, filtered using a 0.22µm syringe filter (Sigma-Aldrich, USA) and centrifuged at 14000 rpm for 10 min at 4°C. The supernatants were collected in glass vials and subjected to UPLC-ESI-QTOF-MS analyses.

2.6.2 LC-MS instrumentation

The determination of bioactive compounds in each sample (*in vivo*-, *in vitro*- derived callus and *in vivo* leaf tissues) was done by using UPLC-ESI-QTOF-MS technique. The analyses were performed by using a 2D nano ACQUITY Ultra-Performance Liquid Chromatography (UPLC) system (Waters Corporation, Milford, USA) online coupled with a SYNAPT G2-Si mass spectrometer (Waters Corporation, Milford, USA) via a NanoLockSpray dual electrospray ionization (ESI) source (Waters Corporation, Milford, USA). The separation of the bioactive compounds was carried out on acquity UPLC BEH C18 column (50x2.1mm, 1.7µm) operated at 35°C.The mobile phase comprised of two solvents: (A) 0.1% formic acid in water, and (B) acetonitrile. Gradient elution was done at a flow rate of 0.3 ml/min at room temperature and elution profile was represented in Table 1. The diode array detector was set at a wavelength range of 214-254 nm and the sample injection volume was 2 µl. The collision gas used was ultrahigh pure nitrogen gas. The mass data were acquired in both positive (+) and negative (-) electrospray ionization (ESI) modes with a scan range from m/z 100 to 1200 Da. The optimized parameters for positive mode were as follows: duration of the run was 25 min including 1766 cycles (0.4 secs each). For MS1 acquisition, ion spray voltage was set to 2500 V; turbo spray temperature of 500°C; collision gas, medium; nebulizer gas (GS1), heater gas (GS2) and curtain gas (CUR) rates were 50, 50 and 25 psi, respectively. For MS2 acquisition, a declustering potential of 80V; collision energy ranges from 20 to 45eV; and collision energy spread of 20V was applied as well. Negative ion mode had the same parameters but with an ion spray voltage of -2500 V.

TABLE 1 Gradient elution profile used in UPLC-ESI-QTOF-MS.

Time (min)	Mobile Phase-A (%)	Mobile Phase-B (%)
0.0	95.0	5.0
2.0	95.0	5.0
10.0	65.0	35.0
16.0	40.0	60.0
18.0	15.0	85.0
22.0	90.0	10.0
25.0	90.0	10.0

2.6.3 Data processing and identification of secondary metabolites

In LC-MS approach, each obtained peak was examined in relation to the Human Metabolome Database (HMDB, http://www.hmdb.ca/) and METLIN (http://metlin.scripps.edu/) databases. In order to identify secondary metabolites, masses were matched within a 500ppm mass accuracy range in the respective libraries. In addition, certain metabolites were identified by comparing the reported MS/MS spectra with their m/z values in the total ion count (TIC) profile. In the positive ionization mode, three parent ion adducts of $[M+H]^+$,$[M+Na]^+$ and $[M+H-H_2O]^+$; and in negative ionization mode, only one parent ion adduct of $[M-H]^-$ were considered in the databases search. The sturdiness of the compounds' identification was validated using a comparison of the fragment masses obtained from the MS-MS spectra of every metabolite.

2.7 Statistical analysis

The *in vitro* culture experiment was conducted in a completely randomized manner in triplicates with six explants per treatment. The biochemical data were also statistically analyzed in triplicates. These data sets were presented as mean ± standard error. The statistical differences were tested using One-way ANOVA, followed by the *post hoc* analysis via Duncan's Multiple Range Test (DMRT) (SPSS software, ver. 26.0) at $p < 0.05$ level.

3 Results

3.1 Callus induction and indirect organogenesis

Firstly, the healthy green leaf tissue of *D. purpurea* was employed as explants for callus induction. The induction was conducted on MS fortified with different concentrations of BAP and 2,4-D. Within about two weeks of culture, earliest signs of callus formation was noticed in all the tested media (Table 2). The calli were observed to be friable, with color varying from milky white to pale yellow (Figures 1A, B). All the tested media produced callus, with an overall rate of callus induction ranging from 94.44% to 22.21%. The best PGR combination was found to be 8.8 µM BAP and 0.9µM 2,4-D in which 94.44% callus induction and 4.9 g fresh biomass were noticed. This was followed by 8.8 µM BAP + 2.3µM 2,4-D and 4.4 µM BAP + 9.0 µM 2,4-D, which induced callus at the rates of 77.77 and 61.10 respectively. The lowest callusing frequency (22.21%) as well as fresh biomass (1.8 grams) was noted at 0.88µM BAP + 2.3µM 2,4-D. The sub-culturing of obtained callus on the same PGR amended MS medium resulted in shoots formation, i.e. indirect shoot organogenesis (Figures 2A, B). All the media

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TABLE 2 Effect of different concentrations of BAP and 2,4-D on callus induction from leaf explants of *D. purpurea* L. after 4 weeks of inoculation.

PGRs Concentration (μM) BAP + 2,4-D	Callusing frequency (%)	Callus biomass (g)
(Control) 0	0^{d}	0^{d}
0.88 + 2.3	22.21 ± 5.56^{c}	1.8 ± 0.7^{c}
2.2 + 4.5	27.77 ± 5.56^{c}	2.6 ± 0.6^{bc}
4.4 + 9.0	61.10 ± 5.55^{b}	3.6 ± 0.8^{ab}
8.8 + 2.3	77.77 ± 11.11^{ab}	4.1 ± 0.5^{ab}
8.8 + 0.9	94.44 ± 9.62^{a}	4.9 ± 0.4^{a}

Each value is represented as mean ± standard error of three repeated experiments (n=6). Mean values with different superscripts within a column are significantly different from each other according to DMRT at $p \leq 0.05$ level.

produced plantlets on callus surface within 3-4 weeks of sub-culturing (Table 3). The highest shoot induction response was found to be 83.33% with maximum 4.7 ± 0.3 shoot numbers/callus mass was noted on BAP (8.8μM) and 2,4-D (0.9μM) added MS medium. On increasing 2,4-D concentrations, the rate of organogenesis gradually reduced. The lowest shoot development ability was observed at 0.88 μM BAP + 2.3μM 2,4-D, showing 11.10% shoot induction rate with 1.5 ± 0.3 mean shoot number/callus mass.

3.2 Biochemical analyses

3.2.1 Total phenolic content

The total phenolic content of MeOH extract of each sample was determined spectrophotometrically and the results are presented in Table 4. The TPC was found to be higher in *in vitro* developed callus and leaf samples than that of *in vivo* leaf sample. *In vitro* derived callus of *D. purpurea* had the highest phenolic content (5.43 ± 0.11 mg GAE/g DW), followed by *in vitro* regenerated leaf extract (4.47 ± 0.04 mg GAE/g DW) and *in vivo* derived leaf (3.64 ± 0.10 mg GAE/g DW). The results indicated that the callus sample accumulated more phenols than the other tested plant tissues.

3.2.2 Total flavonoid content

The total flavonoid contents in different tissue samples of *D. purpurea* were quantified (Table 4). The results were as follows: *in vitro* leaves (3.27 ± 0.01 mg QE/g DW) > leaf derived callus (2.52 ± 0.06 mg QE/g DW) > *in vivo* leaves (1.08 ± 0.16mg QE/g DW). The content of TFC in *D. purpurea* leaves regenerated *in vitro* was the highest, showing a two-fold increment than the *in vivo* grown leaf tissues. The flavonoid content of callus extract was moderate in level.

3.2.3 DPPH radical scavenging activity assay

The methanolic extracts of *in vivo*-, *in vitro* raised leaf and leaf-callus were examined to determine the anti-oxidant potential using the DPPH assay. Results are summarized in Table 5. Both the *in vitro* raised tissues (callus and leaf) exhibited good anti-oxidant activity than the field grown leaf sample. The *in vitro* derived callus revealed the best free radical scavenging activity of 70.68 ± 0.57%, followed by *in vitro* regenerated leaf sample (60.59 ± 0.19%) and field grown leaf tissue (26.67 ± 1.11%). Thus, the lowest scavenging activity was displayed by *in vivo* grown leaf tissue.

3.2.4 Activities of peroxidase and superoxide dismutase

The POD activity of *in vivo*-, *in vitro* grown leaf and leaf -callus sample of *D. purpurea* was assayed. It was observed that the POD activity was the highest in callus sample (0.80 ± 0.03 mM/g FW) than the other tested samples (Table 5). There is a minor difference in peroxidase activity in *in vitro* and *in vivo* derived leaf samples (0.60 ± 0.01mM/g FW and 0.53 ± 0.01 mM/g FW, respectively). The peroxidase activity thus showed this order: leaf derived callus > *in*

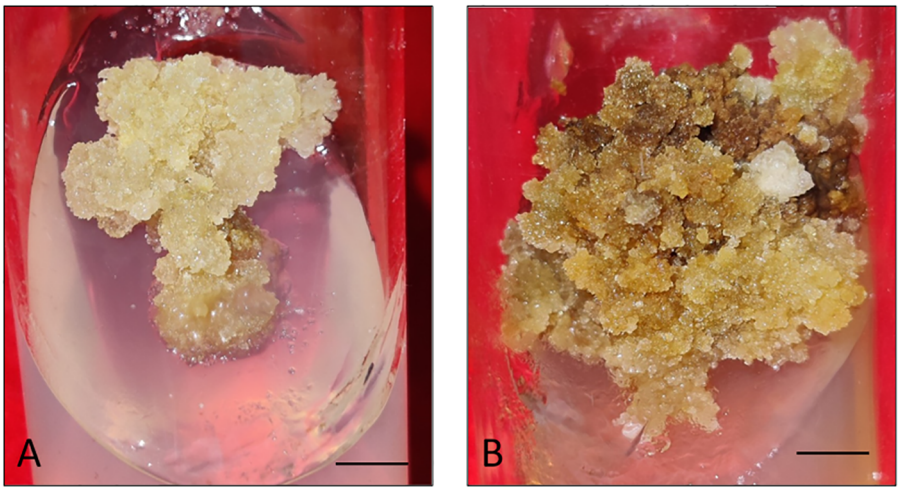

FIGURE 1
(A, B) Callus induction and proliferation from leaf explant of *D. purpurea* L. after 4 weeks of inoculation. (bars= 0.5 cm).

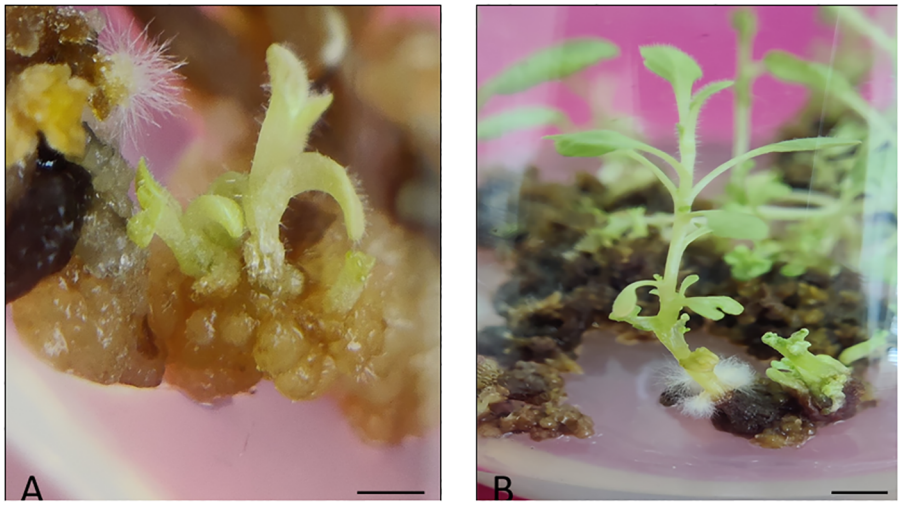

FIGURE 2
(A) Indirect shoot induction from leaf derived callus of *D. purpurea* L. after 4 weeks of sub-culturing (bar= 0.5 cm), **(B)** Elongation of indirect shootlet of *D. purpurea* L. on the same MS medium (bar= 1.0 cm).

vitro raised leaf > *in vivo* grown leaf. SOD assay was conducted to measure the anti-oxidant potential of each sample in *D. purpurea*. In this assay, a significant amount of anti-oxidant activity was noticed in all the extracts. The results revealed that the callus extract showed the maximum NBT degradation activity i.e. 1.52 ± 0.01 Units/mg FW (Table 5), while *in vitro* leaf displayed intermediate response of 1.13 ± 0.01 Units/mg FW, and the lowest activity (0.53 ± 0.02 Units/mg FW) was noted in *in vivo* grown leaf. Thus, the observation suggests that the highest SOD activity was observed in callus extract, followed by *in vitro* - and *in vivo* grown leaf tissue.

3.3 Metabolomics study using untargeted GC-MS approach

Metabolite profiling of methanolic extracts of field grown leaf, *in vitro* raised leaf and leaf-callus of *D. purpurea* were conducted by untargeted GC-MS technique. The phytocompounds in the extracts were identified by several parameters like retention time, fragment ions produced and comparison with the mass spectra of compounds indexed in the NIST library and the WILEY database. The retention time expressed in min, relative peak area percentage (%), molecular formula and molecular weight of compounds detected in each extract were listed in Tables 6–8 and the respective chromatograms are presented in Figure 3. The several compounds found in each extract are known to possess multiple biological activities and belongs to diverse groups of secondary metabolites such as alkaloids, tannins, saponins, fatty acids, terpenoids, flavonoids, sterols etc. The *in vivo*- and *in vitro* grown leaf extract showed the presence of 86 and 77 phytocompounds respectively. Among the compounds detected in methanolic leaf extract grown *in vivo* (Table 6; Figure 3A) are: Palmitic acid (15.09%), Neophytadiene (11.49%), (E)-Phytol (5.62%), linolenic acid (4.84%), stigmasterin (3.85%), 1,11-Undecanediol (3.52%), Phytyl octadecenoate (3.06%), Linoleic acid (2.44%), alpha-tocopherol (2.14%), Gamma-undecanolactone (1.71%), loliolide (0.68%) and others. The methanolic extract of *in vitro* raised leaf tissue of *D. purpurea* displayed a number of phytoconstituents (Table 7; Figure 3B) such as stigmasterin (12.68%), palmitic acid (11.91%), neophytadiene (8.29%), methyl ester of linoleic acid (2.20%), 4-Vinylguaiacol (2.09%), Pyranone (2.04%), Ledol (1.43%), (-)-Cedrenol (0.50%), Glycidyl palmitate (0.28%) etc.

The GC-MS profiling of *D. purpurea* callus extract revealed the presence of 89 bioactive compounds (Table 8; Figure 3C). the major bioactives identified by GC-MS analysis were Stigmasterol (9.30%), 1-Tetratriacontanol heptafluorobutyrate (9.06%), 1,2-Diacetylglycerol (7.50%), Cinnamic acid (5.30%), gamma sitosterol (4.83%), 3-bornanone oxime (4.18%), 3-Hydroxy-5-methoxyflavone (3.62%), Campesterol (2.24%), beta-Amyrin (0.38%), Lauric acid (0.37%) etc. The heatmap and the Venn diagram illustrated in the Figure 4 indicated the common

TABLE 3 Effect of BAP and 2,4-D on indirect organogenesis from leaf and hypocotyl explants of *D. purpurea* L. after 4 weeks of inoculation.

PGRs Concentration (µM) BAP+2,4-D	Indirect organogenesis rate (%)	Mean shoot no./ callus mass
(Control) 0	0^{d}	0^{d}
0.88 + 2.3	11.10 ± 5.55^{d}	1.5 ± 0.3^{c}
2.2 + 4.5	33.33 ± 9.62^{c}	2.1 ± 0.7^{c}
4.4 + 9.0	38.89 ± 5.56^{c}	2.5 ± 0.8^{bc}
8.8 + 2.3	61.10 ± 5.55^{b}	3.8 ± 0.1^{ab}
8.8 + 0.9	83.33 ± 9.62^{a}	4.7 ± 0.3^{a}

Each value is represented as mean ± standard error of three repeated experiments (n=6). Mean values with different superscripts within a column are significantly different from each other according to DMRT at $p \leq 0.05$ level.

TABLE 4 Total phenolic content (TPC) and total flavonoid content (TFC) of callus and leaf tissues of *D. purpurea* L.

Sample	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
In vivo leaf	3.64 ± 0.10^{c}	1.08 ± 0.16^{c}
In vitro leaf	4.47 ± 0.04^{b}	3.27 ± 0.01^{a}
Leaf derived callus	5.43 ± 0.11^{a}	2.52 ± 0.06^{b}

TPC: total phenolic content, TFC, total flavonoid content; GAE, gallic acid equivalent; QE, quercetin equivalent; DW, dry weight. Each value is represented as mean ± standard error of three repeated experiments. Mean values with different superscripts within a column are significantly different from each other according to DMRT at $p \leq 0.05$ level.

phytocompounds present in all the three or two samples examined. In total, 5 metabolites had been found to be present in all the three extracts, these were 4-Vinylguaiacol, dihydro methyl jasmonate, Neophytadiene, hexamethyl-pyranoindane, (E)-phytol; whereas 13 bioactives were found to be common in both *in vivo*- and *in vitro* derived leaf tissue. There are 5 secondary metabolites present in field grown leaf and leaf derived callus, while only 3 phytocompounds were common in leaf-callus and *in vitro* raised leaf tissue.

3.4 LC-MS based metabolites profiling

Using UPLC-ESI-QTOF-MS method, the phytochemicals of field- and laboratory grown leaf and leaf-callus of *D. purpurea* were investigated in both the positive and negative ionization modes. The base peak chromatograms (positive and negative ion modes) of each sample are presented in Figures 5–10 with the retention time and measured m/z of the compounds. The phytocompounds were identified by comparing the $[M+H]^+$, $[M+Na]^+$ and $[M+H-H_2O]^+$ protonated molecule (for positive mode) and $[M-H]^-$ de-protonated molecule (for negative mode) and their fragmentation in MS/MS spectra with the data recorded in the HMDB and METLIN databases. The identified phytocompounds were tabulated in Tables 9–14 with their retention time, measured m/z, exact m/z, molecular formula and product ions (ms/ms).

TABLE 5 DPPH, POD and SOD activities of *in vivo*-, *in vitro*- leaf and callus samples of *D. purpurea* L.

Sample	DPPH Scavenging activity (%)	POD assay (mM/g FW)	SOD assay (U/mg FW)
In vivo leaf	26.67 ± 1.11^{c}	0.53 ± 0.01^{c}	0.53 ± 0.02^{c}
In vitro leaf	60.59 ± 0.19^{b}	0.60 ± 0.01^{b}	1.13 ± 0.01^{b}
Leaf derived callus	70.68 ± 0.57^{a}	0.80 ± 0.03^{a}	1.52 ± 0.01^{a}

DPPH, 2,2-diphenyl-1-picrylhydrazyl; POD, peroxidase; SOD, superoxide dismutase; FW, fresh weight. Each value is represented as mean ± standard error of three repeated experiments. Mean values with different superscripts within a column are significantly different from each other according to DMRT at $p \leq 0.05$ level.

3.4.1 Analyses in the positive-ion mode

The *in vivo* grown leaf sample contained a total of 20 metabolites in 40 min elution time, mostly of which were flavonoids and fatty acyl groups. The first eluted compound was mesoxalic acid with a mass of 119 m/z at 0.67 min (Table 9; Figure 5). Various flavonoids such as apigenin-7-sulfate (6.08 min), luteolin (6.84 min) and kaempferide (7.39 min) were identified with the mass of 372.99 m/z, 287.05 m/z and 301.07 m/z, respectively. Also, certain fatty acyls like methyl linolenate (1.08 min), octyl octanoate (9.70 min) and hexadecanamide (11.08 min) were detected having masses of 275.23 m/z, 257.24 m/z and 278.24 m/z, respectively. An important cardenolide (20,22-Dihydrodigoxigenin) was also found in *in vivo* leaf extract at 13.02 min with the mass of 415.24 m/z. The LC-MS analysis of *in vitro* leaf extract of *D. purpurea* revealed the presence of 17 phytocompounds of diverse classes like carbohydrates, fatty acyls, flavonoids, tannins etc. (Table 10; Figure 6). The first compound showed the characteristic protonated $[M+Na]^+$ molecule at m/z 121.06, corresponding to hexenal ($C_6H_{10}O$), other major compounds detected were rosmarinic acid (polyphenol), digoxigenin monodigitoxoside (a steroidal saponin), methyl 4,6-di-O-galloyl-beta-D-glucopyranoside (a tannin) and others. One coumarin derivative named sagecoumarin ($C_{27}H_{20}O_{12}$) was also detected at 11.86 min with a mass of 519.09 m/z. On the contrary, the methanolic extract of leaf-callus indicated the presence of only 6 phytoconstituents (Table 11; Figure 7). Among the compounds identified, 3 compounds belong to flavonoid group, 2 from steroidal group and one from terpenoid class. The compounds successfully identified were: Epicatechin 3-glucoside (453.13 m/z, $[M+H]^+$); (15a,20R)-Dihydroxypregn-4-en-3-one 20-[glucosyl-(1->4)-6-acetyl-glucoside] (681.34 m/z, $[M+H-H_2O]^+$); Quercetin 3-glucoside 7-xyloside (597.14 m/z, $[M+H]^+$); stigmasterol 3-O-beta-D-glucoside (597.41 m/z, $[M+Na]^+$); Erythrodiol 3-decanoate (597.52 m/z, $[M+H]^+$) and 3',4',5'-Trimethoxyflavone (313.10 m/z, $[M+H]^+$).

3.4.2 Analyses in the negative-ion mode

Similar to positive ion, all the three samples were analyzed in negative ionization mode. Firstly, the field grown leaf sample was investigated and it showed some 22 bioactive compounds, more than observed in positive ion mode. The first compound eluted was 3,3',4',7-Tetrahydroxyflavan (flavonoid) at 0.70 min with the mass 273.07 m/z (Table 12; Figure 8). Likewise, multiple flavonoids such as epicatechin 3-glucoside (3.49 min), nepitrin (4.87 min), apigenin (7.35 min) were detected with the masses of 451.12 m/z, 477.10 m/z and 269.04 m/z, respectively. A base peak at m/z 425 was noticed, corresponding to the alpha-amyrin (a triterpenoid) at 5.21 min. Further analyses revealed that methanolic leaf (*in vivo*) extract contained several phytocompounds involving fatty acyls, steroids, triacyl glycerols, lignans and benzenoids. In a similar manner, the *in vitro* regenerated leaf extract was known to possess 16 phytoconstituents belonging to varied metabolite classes. 2-Hydroxypropyl glucosinolate (376.03 m/z), nepitrin (477.10 m/z), rhamnetin 3-laminaribioside (639.15 m/z), pregn-5-ene-3,20-dione-17-ol (329.21 m/z) and 2,3-Diphosphoglyceric acid (264.95

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TABLE 6 List of phytocompounds identified in the methanolic extract of *in vivo* grown leaf tissue of *D. purpurea* L. by GC-MS.

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
1	6.814	0.21	2-trimethylsilyl-1,3-dithiane	$C_7H_{16}S_2Si$	192
2	9.457	0.19	trimethyl-tetrahydronaphthalene	$C_{13}H_{18}$	174
3	10.182	0.55	5-methyl-2-isopropylphenol	$C_{10}H_{14}O$	150
4	10.559	0.77	4-vinylguaiacol	$C_9H_{10}O_2$	150
5	10.780	0.17	1,1,5,6-tetramethylindane	$C_{13}H_{18}$	174
6	11.084	0.83	3-cyclohexene-1,1-dimethanol	$C_8H_{14}O_2$	142
7	11.415	1.08	9-oxa-bicyclo [3.3.1] nonane-2,7-diol	$C_8H_{14}O_3$	158
8	11.617	0.52	tetradecanolactone	$C_{14}H_{26}O_2$	226
9	11.963	3.52	1,11-undecanediol	$C_{11}H_{24}O_2$	188
10	12.082	1.71	gamma-undecanolactone	$C_{11}H_{20}O_2$	184
11	12.351	0.22	glutaric acid, di(myrtenyl) ester	$C_{25}H_{36}O_4$	400
12	12.552	0.44	succinic acid, tridec-2-yn-1-yl 2-methylpentyl ester	$C_{23}H_{40}O_4$	380
13	12.855	0.23	4-butoxyaniline	$C_{10}H_{15}NO$	165
14	12.978	1.15	1-[(2z)-2-ethylidene-1-hydroxycyclohexyl] ethanone	$C_{10}H_{16}O_2$	168
15	13.350	1.55	1,6-anhydro-beta-d-glucopyranose	$C_6H_{10}O_5$	162
16	13.449	0.23	globulol	$C_{15}H_{26}O$	222
17	13.647	0.38	lauric acid	$C_{12}H_{24}O_2$	200
18	13.792	0.09	2,2,4-trimethylpentanediol-1,3-diisobutyrate	$C_{16}H_{30}O_4$	286
19	14.229	0.14	3-hydroxy-beta-damascone	$C_{13}H_{20}O_2$	208
20	14.380	0.52	megastigmatrienone	$C_{13}H_{18}O$	190
21	14.592	0.59	dihydro methyl jasmonate	$C_{13}H_{22}O_3$	226
22	14.650	0.16	3-oxo-alpha-ionol	$C_{13}H_{20}O_2$	208
23	14.710	0.35	dihydroselarene	$C_{20}H_{34}$	274
24	14.745	0.29	beta-methylionone	$C_{14}H_{22}O$	206
25	15.252	0.47	1-heptadec-1-ynyl-cyclohexanol	$C_{23}H_{42}O$	334
26	15.709	1.33	2-hexylcinnamaldehyde	$C_{15}H_{20}O$	216
27	15.857	0.81	myristic acid	$C_{14}H_{28}O_2$	228
28	16.040	0.08	3-phenylpropanal	$C_9H_{10}O$	134
29	16.227	0.68	loliolide	$C_{11}H_{16}O_3$	196
30	16.372	0.39	isopropyl tetradecanoate	$C_{17}H_{34}O_2$	270
31	16.497	11.49	neophytadiene	$C_{20}H_{38}$	278
32	16.585	0.61	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268
33	16.700	0.78	hexamethyl-pyranoindane	$C_{18}H_{26}O$	258
34	16.750	3.42	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
35	16.895	0.03	adipic acid, pentyl propyl ester	$C_{14}H_{26}O_4$	258
36	16.943	5.62	(e)-phytol	$C_{20}H_{40}O$	296
37	17.145	0.40	eicosanoic acid, phenylmethyl ester	$C_{27}H_{46}O_2$	402
38	17.423	0.25	hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270

(Continued)

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TABLE 6 Continued

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
39	17.535	0.17	octadecyl chloride	$C_{18}H_{37}Cl$	288
40	17.705	0.56	2,6-dimethyltridecanenitrile	$C_{15}H_{29}N$	223
41	17.905	15.09	palmitic acid	$C_{16}H_{32}O_2$	256
42	18.142	0.57	2-hexyl-1-decanol	$C_{16}H_{34}O$	242
43	18.953	0.20	cis-1-chloro-9-octadecene	$C_{18}H_{35}Cl$	286
44	19.065	0.25	2-methyltetracosane	$C_{25}H_{52}$	352
45	19.225	5.39	3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	296
46	19.520	2.44	linoleic acid	$C_{18}H_{32}O_2$	280
47	19.583	4.84	linolenic acid	$C_{18}H_{30}O_2$	278
48	19.766	1.71	stearic acid	$C_{18}H_{36}O_2$	284
49	20.254	0.16	methyl 8-(3-octyl-2-oxiranyl) octanoate	$C_{19}H_{36}O_3$	312
50	20.436	0.55	methyl-1-isopropyl-7-phenanthrene	$C_{18}H_{18}$	234
51	20.872	0.21	glycidyl palmitate	$C_{19}H_{36}O_3$	312
52	21.106	0.78	9-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
53	21.296	0.60	n-eicosylcyclohexane	$C_{26}H_{52}$	364
54	21.358	0.72	delta-tridecalactone	$C_{13}H_{24}O_2$	212
55	21.499	0.25	eicosanoic acid	$C_{20}H_{40}O_2$	312
56	21.638	0.46	5-methyl-4-(prop-1-en-2-ylsulfanyl)-2h,3h-cyclopenta[a]naphthalen-1-one	$C_{17}H_{16}OS$	268
57	21.920	0.07	methyl 18-oxidanyloctadeca-9,12-dienoate, tms derivative	$C_{22}H_{42}O_3Si$	382
58	21.960	0.31	11-tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	254
59	22.115	0.23	2-hydroxyethyl stearate	$C_{20}H_{40}O_3$	328
60	22.264	0.30	heptadecyl octanoate	$C_{25}H_{50}O_2$	382
61	22.478	0.33	2-hexyldecanol	$C_{16}H_{34}O$	242
62	22.700	0.49	propanoic acid, pentafluoro-, 1-phenyl-1,2-ethanediyl ester	$C_{14}H_8F_{10}O_4$	430
63	22.770	0.08	9-decenyl laurate	$C_{22}H_{42}O_2$	338
64	23.273	0.16	dimethyl 2-(2-piperidinylidene) malonate	$C_{10}H_{15}NO_4$	213
65	23.645	0.38	8s,14-cedrandiol	$C_{15}H_{26}O_2$	238
66	23.795	0.30	octadecyl 2-ethylhexanoate	$C_{26}H_{52}O_2$	396
67	23.845	0.17	linoleyl acetate	$C_{20}H_{36}O_2$	308
68	23.975	0.18	stearyl monoglyceride	$C_{21}H_{44}O_3$	344
69	24.062	0.39	methyl dehydroabietate	$C_{21}H_{30}O_2$	314
70	24.364	1.00	cholest-14-en-3-ol	$C_{27}H_{46}O$	386
71	24.497	0.24	phytyl heptadecanoate	$C_{37}H_{72}O_2$	548
72	24.919	0.23	squalene	$C_{30}H_{50}$	410
73	25.235	0.40	alpha-tocospiro b	$C_{29}H_{50}O_4$	462
74	25.420	1.25	alpha-tocospiro a	$C_{29}H_{50}O_4$	462
75	25.690	0.39	4-methylcyclohexanone semicarbazone	$C_8H_{15}N_3O$	169
76	26.309	0.60	2-octyl-3-pentadecyloxirane	$C_{25}H_{50}O$	366

(Continued)

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TABLE 6 Continued

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
77	27.123	0.33	retinol	$C_{20}H_{30}O$	286
78	27.450	0.11	3-bromo-1-propanol, tms derivative	$C_6H_{15}BrOSi$	210
79	27.519	0.47	5,5-diethylpentadecane	$C_{19}H_{40}$	268
80	27.891	0.29	22,23-dihydroergosterol	$C_{28}H_{46}O$	398
81	28.658	2.14	d-alpha-tocopherol	$C_{29}H_{50}O_2$	430
82	28.868	1.07	phytyl stearate	$C_{38}H_{74}O_2$	562
83	30.913	3.85	stigmasterin	$C_{29}H_{48}O$	412
84	32.075	0.05	dihydropleurotin	$C_{21}H_{24}O_5$	356
85	32.745	3.93	(e)-3,7,11,15-tetramethylhexadec-2-en-1-yl decanoate	$C_{30}H_{58}O_2$	450
86	38.687	3.06	phytyl octadecanoate	$C_{38}H_{74}O_2$	562

TABLE 7 List of phytocompounds identified in the methanolic extract of *in vitro* grown leaf tissue of *D. purpurea* L. by GC-MS.

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
1	8.156	2.04	pyranone	$C_6H_8O_4$	144
2	10.193	1.41	5-methyl-2-isopropylphenol	$C_{10}H_{14}O$	150
3	10.560	2.09	4-vinylguaiacol	$C_9H_{10}O_2$	150
4	11.575	0.63	2-[2-(alpha-hydroxybenzyl) propylidene]-1,3-oxathiane	$C_{14}H_{18}O_2S$	250
5	12.160	1.71	2-propanone, 2-propenylhydrazone	$C_6H_{12}N_2$	112
6	12.445	0.17	o-tert-butylphenol	$C_{10}H_{14}O$	150
7	12.568	1.43	ledol	$C_{15}H_{26}O$	222
8	12.700	2.79	2-tridecynyl 2,6-difluorobenzoate	$C_{20}H_{26}F_2O_2$	336
9	12.999	0.14	7-epi-sesquithujene	$C_{15}H_{24}$	204
10	13.467	0.50	1-hydroxy-6-(3-isopropenyl-cycloprop-1-enyl)-6-methyl-heptan-2-one	$C_{14}H_{22}O_2$	222
11	13.745	0.10	n,n-di(2-cyclohexen-1-yl)amine	$C_{12}H_{19}N$	177
12	13.795	0.22	2-propyltetrahydrofuran	$C_7H_{14}O$	114
13	14.355	0.55	2-bornyl valerate	$C_{15}H_{26}O_2$	238
14	14.539	0.10	(-)-alpha-terpineol	$C_{10}H_{18}O$	154
15	14.592	1.71	dihydro methyl jasmonate	$C_{13}H_{22}O_3$	226
16	14.744	1.62	beta-methylionone	$C_{14}H_{22}O$	206
17	14.790	0.13	4-(1,1,3,3-tetramethylbutyl) phenol	$C_{14}H_{22}O$	206
18	14.836	0.27	1-(4-isopropylphenyl)-2-methylpropyl acetate	$C_{15}H_{22}O_2$	234
19	15.183	0.22	1,3-dimethyladamantane	$C_{12}H_{20}$	164
20	15.253	0.85	1-heptadec-1-ynyl-cyclohexanol	$C_{23}H_{42}O$	334
21	15.714	0.36	1-isobutyl-4-(1-methyl-2-propenyl) benzene	$C_{14}H_{20}$	188
22	15.858	0.38	n-tridecoic acid	$C_{13}H_{26}O_2$	214
23	15.949	0.50	(-)-cedrenol	$C_{15}H_{26}O$	222
24	16.496	8.29	neophytadiene	$C_{20}H_{38}$	278

(Continued)

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TABLE 7 Continued

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
25	16.703	0.45	hexamethyl-pyranoindane	$C_{18}H_{26}O$	258
26	16.749	2.20	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
27	16.943	3.00	(e)-phytol	$C_{20}H_{40}O$	296
28	17.027	0.19	decanol	$C_{10}H_{22}O$	158
29	17.428	0.24	octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
30	17.891	11.91	palmitic acid	$C_{16}H_{32}O_2$	256
31	18.140	0.62	3-hydroxypropyl palmitate, tms derivative	$C_{22}H_{46}O_3Si$	386
32	18.202	0.17	glutaric acid, dodec-2-en-1-yl tetradecyl ester	$C_{31}H_{58}O_4$	494
33	18.486	0.35	1,1,2,3,3-pentachloropropane	$C_3H_3Cl_5$	214
34	19.069	0.61	heneicosane	$C_{21}H_{44}$	296
35	19.199	0.45	cis-1-chloro-9-octadecene	$C_{18}H_{35}Cl$	286
36	19.229	0.49	3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	296
37	19.342	0.19	arachidic acid methyl ester	$C_{21}H_{42}O_2$	326
38	19.449	0.51	20-(5-methyltetrahydro-2h-pyran-2yl) pregnane	$C_{27}H_{46}O$	386
39	19.515	2.20	linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294
40	19.577	2.05	linolenic acid	$C_{18}H_{30}O_2$	278
41	19.766	1.81	stearic acid	$C_{18}H_{36}O_2$	284
42	19.903	0.32	3,5-di-t-butyl-4-hydroxyanisole	$C_{15}H_{24}O_2$	236
43	20.010	0.53	3,5-dimethoxycyclohexanol	$C_8H_{16}O_3$	160
44	20.115	0.09	7,7-dimethoxyheptanal	$C_9H_{18}O_3$	174
45	20.443	1.18	methyl-1-isopropyl-7-phenanthrene	$C_{18}H_{18}$	234
46	20.555	0.16	14-heptacosanone	$C_{27}H_{54}O$	394
47	20.774	0.66	dimethylaminoethyl palmitate	$C_{20}H_{41}NO_2$	327
48	20.874	0.28	glycidyl palmitate	$C_{19}H_{36}O_3$	312
49	20.967	0.26	2-pyrrolidoneacetamide	$C_6H_{10}N_2O_2$	142
50	21.109	0.31	fumaric acid, dec-4-enyl tridecyl ester	$C_{27}H_{48}O_4$	436
51	21.167	0.54	1-benzothiophene-3-carboxylic acid	$C_9H_6O_2S$	178
52	21.295	0.30	octyl octanoate	$C_{16}H_{32}O_2$	256
53	21.361	0.39	phytyl decanoate	$C_{30}H_{58}O_2$	450
54	21.490	0.19	chloromethyl 6-chloroheptanoate	$C_8H_{14}Cl_2O_2$	212
55	21.635	0.47	neoergosterol	$C_{27}H_{40}O$	380
56	22.037	0.18	2-(1-undecyl) benzene-1,3-dicarbonitrile	$C_{23}H_{34}O_2$	342
57	22.180	1.10	dimethylaminoethyl oleate	$C_{22}H_{43}NO_2$	353
58	22.261	1.21	n-methyl-n,n-bis(3-aminopropyl)amine	$C_7H_{19}N_3$	145
59	22.307	0.67	2-(heptyloxy)-4,6-dimethyl-1,3,2-dioxaborinane	$C_{12}H_{25}BO_3$	228
60	22.525	1.10	ammodendrine	$C_{12}H_{20}N_2O$	208
61	22.707	0.55	nonanoic acid, hexyl ester	$C_{15}H_{30}O_2$	242
62	22.766	0.17	pentadecyl nonanoate	$C_{24}H_{48}O_2$	368

(Continued)

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TABLE 7 Continued

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
63	23.310	0.32	dehydroabietic acid, methyl ester	$C_{22}H_{32}O_2$	328
64	23.712	1.19	1-bromotriacontane	$C_{30}H_{61}Br$	500
65	23.797	0.63	4-methyloctadecanoic acid	$C_{19}H_{38}O_2$	298
66	23.904	0.55	1-chloro-1-(3,3-diethoxy-1-propynyl)-2,2,3,3-tetramethylcyclopropane	$C_{14}H_{23}ClO_2$	258
67	23.980	2.31	13-methylheptacosane	$C_{28}H_{58}$	394
68	24.064	1.57	dehydroabietic acid	$C_{21}H_{30}O_2$	314
69	24.310	0.11	2-chloro-4-dodecylphenol	$C_{18}H_{29}ClO$	296
70	24.921	1.89	squalene	$C_{30}H_{50}$	410
71	25.313	2.32	tetrapentacontane	$C_{54}H_{110}$	758
72	25.671	1.22	tetracosane	$C_{24}H_{50}$	338
73	26.745	0.16	octyl 12-hydroxyoctadecanoate	$C_{26}H_{52}O_3$	412
74	28.658	6.52	alpha-tocopherol	$C_{29}H_{50}O_2$	430
75	30.429	0.53	(-)-beta-sitosterol	$C_{29}H_{50}O$	414
76	30.923	12.68	stigmasterin	$C_{29}H_{48}O$	412
77	32.179	1.68	gamma sitosterol	$C_{29}H_{50}O$	414

TABLE 8 List of phytocompounds identified in the methanolic extract of leaf derived callus of *D. purpurea* L. by GC-MS.

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
1	6.090	1.26	pyranone	$C_6H_8O_4$	144
2	6.653	0.37	n-ethyl-n-methyl-2-propen-1-amine	$C_6H_{13}N$	99
3	6.854	0.28	decanal	$C_{10}H_{20}O$	156
4	7.060	0.80	prenyl isobutyrate	$C_9H_{16}O_2$	156
5	7.468	0.43	linalool acetate	$C_{12}H_{20}O_2$	196
6	7.539	0.30	geraniol	$C_{10}H_{18}O$	154
7	7.615	0.16	1-monoacetin	$C_5H_{10}O_4$	134
8	7.690	0.43	beta-cyclocitral	$C_{10}H_{16}O$	152
9	8.042	1.82	1-ethylcyclohexanol	$C_8H_{16}O$	128
10	8.495	0.07	4-vinylguaiacol	$C_9H_{10}O_2$	150
11	8.595	0.20	4-deoxypyridoxine	$C_8H_{11}NO_2$	153
12	8.758	7.50	1,2-diacetylglycerol	$C_7H_{12}O_5$	176
13	8.834	0.22	pulegone	$C_{10}H_{16}O$	152
14	9.025	0.08	eugenol	$C_{10}H_{12}O_2$	164
15	9.118	0.08	2-methyl undecanal	$C_{12}H_{24}O$	184
16	9.361	1.89	9-oxabicyclo [3.3.1] nonane-2,6-diol	$C_8H_{14}O_3$	158
17	9.716	1.61	4-ethylcatechol	$C_8H_{10}O_2$	138
18	9.998	0.15	1,1-dimethyl-3-methylene-2-vinylcyclohexane	$C_{11}H_{18}$	150

(Continued)

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TABLE 8 Continued

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
19	10.050	0.62	3-octadecenal	$C_{18}H_{34}O$	266
20	10.282	5.30	cinnamic acid	$C_9H_8O_2$	148
21	10.624	2.16	4-hydroxy-2,4,5-trimethyl-2,5-cyclohexadien-1-one	$C_9H_{12}O_2$	152
22	10.873	0.24	ethyl propylphosphonofluoridate	$C_5H_{12}FO_2P$	154
23	11.002	0.61	5-hydroxy-4,7,7-trimethylbicyclo [2.2.1] heptan-2-one	$C_{10}H_{16}O_2$	168
24	11.214	1.54	6-dodecanol	$C_{12}H_{26}O$	186
25	11.313	0.18	jasmone	$C_{11}H_{16}O$	164
26	11.425	4.15	2-methylenecyclohexanol	$C_7H_{12}O$	112
27	11.562	0.37	delta.1,9-10-methyl-2-octalone	$C_{11}H_{16}O$	164
28	11.636	0.65	decyl formate	$C_{11}H_{22}O_2$	186
29	11.708	0.37	lauric acid	$C_{12}H_{24}O_2$	200
30	11.846	0.31	ethyl 2,3-nonadienoate	$C_{11}H_{18}O_2$	182
31	12.525	0.29	tridecanedial	$C_{13}H_{24}O_2$	212
32	12.707	1.39	dihydro methyl jasmonate	$C_{13}H_{22}O_3$	226
33	12.898	0.43	1-(4-isopropylphenyl)-2-methylpropyl acetate	$C_{15}H_{22}O_2$	234
34	13.398	0.22	stearyl acetate	$C_{20}H_{40}O_2$	312
35	13.951	0.13	tridecanoic acid	$C_{13}H_{26}O_2$	214
36	14.100	4.18	3-bornanone oxime	$C_{10}H_{17}NO$	167
37	14.594	1.72	4-(2,2,6-trimethylbicyclo [4.1.0] hept-1-yl)-2-butanone	$C_{14}H_{24}O$	208
38	14.675	0.08	neophytadiene	$C_{20}H_{38}$	278
39	14.744	0.12	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268
40	14.872	0.35	hexamethyl-pyranoindane	$C_{18}H_{26}O$	258
41	15.122	0.15	4,8,13-duvatriene-1,3-diol	$C_{20}H_{34}O_2$	306
42	15.222	0.16	methyl 4-o-benzyl-alpha-l-rhamnopyranoside	$C_{14}H_{20}O_5$	268
43	15.593	0.45	methyl palmitate	$C_{17}H_{34}O_2$	270
44	16.042	1.37	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256
45	16.190	0.23	trehalose	$C_{12}H_{22}O_{11}$	342
46	16.255	0.09	trans-10-phenyl-2-decalone	$C_{16}H_{20}O$	228
47	16.299	0.11	methyl pentacosanoate	$C_{26}H_{52}O_2$	396
48	16.726	0.32	octyl hexopyranoside	$C_{14}H_{28}O_6$	292
49	17.160	0.23	1-nonadecanol	$C_{19}H_{40}O$	284
50	17.284	0.70	methyl elaidate	$C_{19}H_{36}O_2$	296
51	17.390	0.35	(e)-phytol	$C_{20}H_{40}O$	296
52	19.039	0.11	heptadecyl bromide	$C_{17}H_{35}Br$	318
53	19.260	0.23	oleic anhydride	$C_{36}H_{66}O_3$	546
54	19.460	0.14	n-tetradecylcyclohexane	$C_{20}H_{40}$	280
55	19.525	0.17	methyl dehydroabietate	$C_{21}H_{30}O_2$	314
56	19.659	0.30	5,7-dihydroxyflavone	$C_{15}H_{10}O_4$	254

(Continued)

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TABLE 8 Continued

S.No.	RT (min)	Peak Area %	Name of the Compound	Molecular Formula	Molecular Weight
57	19.789	0.57	alpha-monostearin	$C_{21}H_{42}O_4$	358
58	19.956	0.23	podocarpa-8,11,13-trien-3-one	$C_{17}H_{22}O$	242
59	20.430	0.06	3-(3',4'-dimethoxyphenyl) coumarin	$C_{17}H_{14}O_4$	282
60	20.544	3.62	3-hydroxy-5-methoxyflavone	$C_{16}H_{12}O_4$	268
61	20.780	0.42	1,5-dimethoxyanthraquinone	$C_{16}H_{12}O_4$	268
62	20.852	0.55	2-monopalmitin	$C_{19}H_{38}O_4$	330
63	21.111	2.60	1,5-dimethoxyanthra-9,10-quinone	$C_{16}H_{12}O_4$	268
64	21.202	0.70	4,22-cholestadien-3-one	$C_{27}H_{42}O$	382
65	21.410	0.25	epi-allogibberinic acid	$C_{18}H_{20}O_3$	284
66	21.558	0.22	2-hexyldecanol	$C_{16}H_{34}O$	242
67	21.767	0.21	3-methylcholanthren-2-ol	$C_{21}H_{16}O$	284
68	22.140	0.15	1-eicosene	$C_{20}H_{40}$	280
69	22.218	1.61	1-octacosanol	$C_{28}H_{58}O$	410
70	22.400	1.05	3-methoxyestra-1(10),2,4,6,8-pentaen-17-ol	$C_{19}H_{22}O_2$	282
71	22.855	0.22	1-nonacosene	$C_{29}H_{58}$	406
72	22.930	0.54	methyl tetratriacontyl ether	$C_{35}H_{72}O$	508
73	23.011	9.06	1-tetratriacontanol, heptafluorobutyrate	$C_{38}H_{69}F_7O_2$	690
74	23.342	0.23	2-methylhexacosane	$C_{27}H_{56}$	380
75	23.418	0.66	3,17,20,21-tetrahydroxypregnan-11-one	$C_{21}H_{34}O_5$	366
76	23.623	2.08	heneicosyl trifluoroacetate	$C_{23}H_{43}F_3O_2$	408
77	24.555	0.93	stigmasta-4,7,22-trien-3-beta-ol	$C_{29}H_{46}O$	410
78	24.806	0.38	beta-amyrin	$C_{30}H_{50}O$	426
79	25.030	0.62	22,23-dihydroergosterol	$C_{28}H_{46}O$	398
80	25.193	1.78	methyl commate a	$C_{32}H_{52}O_4$	500
81	25.465	0.43	5-cholesten-3-beta-ol	$C_{27}H_{46}O$	386
82	26.462	2.24	campesterol	$C_{28}H_{48}O$	400
83	26.730	9.30	stigmasterol	$C_{29}H_{48}O$	412
84	27.401	4.83	gamma sitosterol	$C_{29}H_{50}O$	414
85	27.580	0.51	isofucosterol	$C_{29}H_{48}O$	412
86	27.777	1.60	7,22-ergostadienone	$C_{28}H_{44}O$	396
87	28.400	0.25	cycloartenol	$C_{30}H_{50}O$	426
88	28.550	1.30	3,5-cholestadien-7-one	$C_{27}H_{42}O$	382
89	29.183	2.58	24-methylcycloartenol	$C_{31}H_{52}O$	440

m/z) were some of the major compounds (Table 13; Figure 9). Finally, the callus extract of *D. purpurea* was also analyzed in negative ion mode and 11 bioactives were noted (Table 14; Figure 10). The characteristic compounds identified were squamoxinone C (fatty acyl) at 8.00 min, Delta4,16-Pregnadiene-3,20-dione at (steroid) at 10.29 min, 2,3-Diphosphoglyceric acid (carbohydrate) at 12.96 min with the masses corresponding to 595.45 m/z, 311.20 m/z and 264.95 m/z, respectively. Overall, the LC-MS analysis (both positive and negative ion mode) of the field grown leaf sample of *D. purpurea* had shown the presence of flavonoids in the major proportion (28.57%), followed by fatty acyl groups (19.04%), steroids (9.52%), triterpenoids (4.76%) and other compounds (26.21%) viz. the monosaccharide, saturated hydrocarbon, benzenoid etc. (Figure 11A). The *in vitro* raised leaf extract had the flavonoids as the highest occurring bioactive group (21.21%), followed by fatty acyls (18.18%), carbohydrates (9.09%),

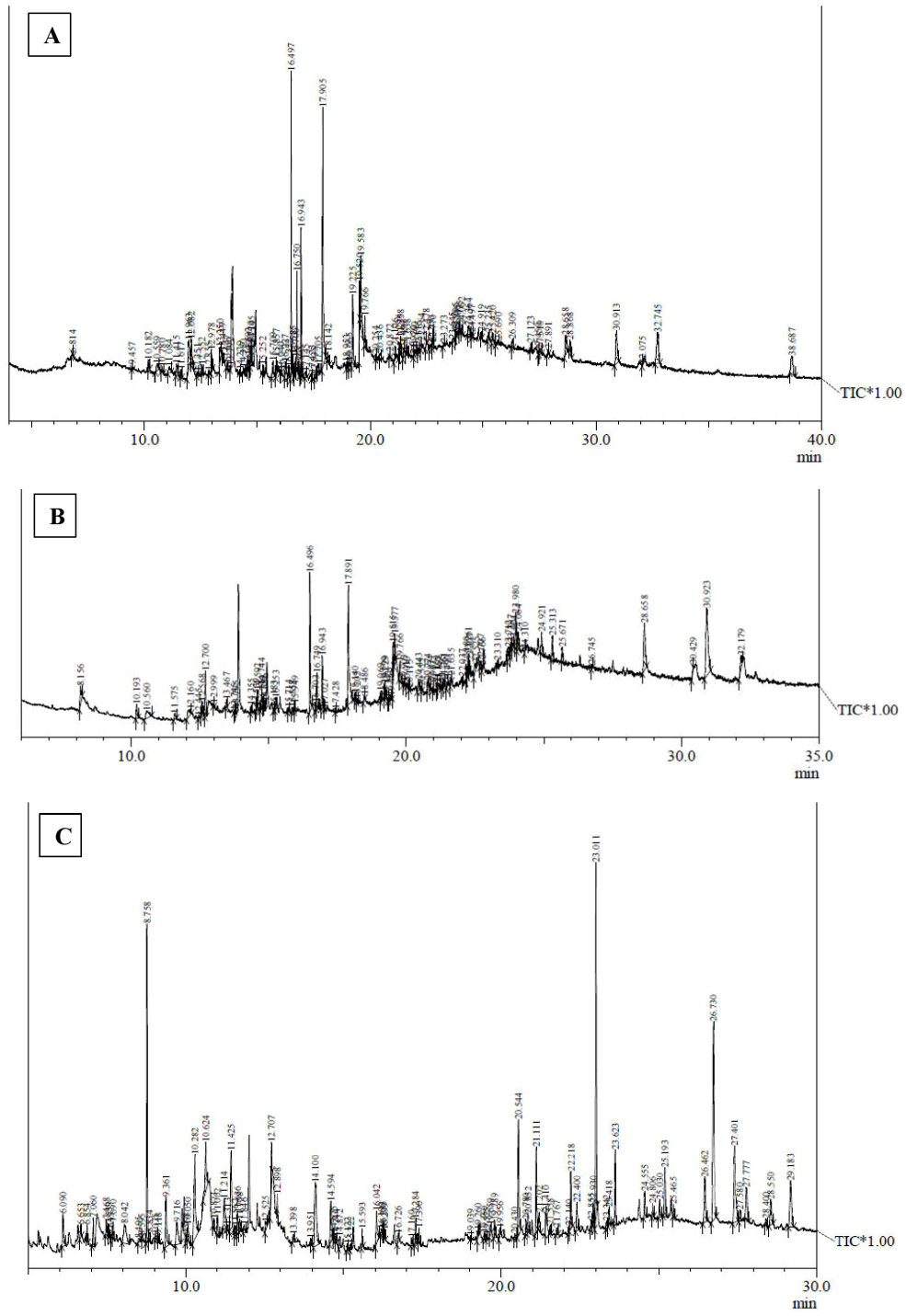

FIGURE 3
GC-MS chromatograms of methanolic extracts of **(A)** *in vivo* grown leaf tissue, **(B)** *in vitro* grown leaf tissue, and **(C)** leaf derived callus of *D. purpurea* L.

carbonyl compounds, carboxylic acids, coumarins and steroids (all at 6.06%), tannins (3.03%) etc. (Figure 11B). The major metabolite groups noted in callus extract followed the same trend as was noticed *in vivo*- and *in vitro* grown leaf tissue, with flavonoid being the top group (29.42%), then fatty acyl (17.65%), steroids (17.65%), triterpenoids (11.76%) and tannins (5.88%) (Figure 11C).

4 Discussion

To the best of our knowledge, this is the first study in characterizing metabolomes of cultured tissues in *Digitalis purpurea* by employing untargeted GC-MS and LC-MS approaches. Thus, in the current study, the leaf explants of *D.*

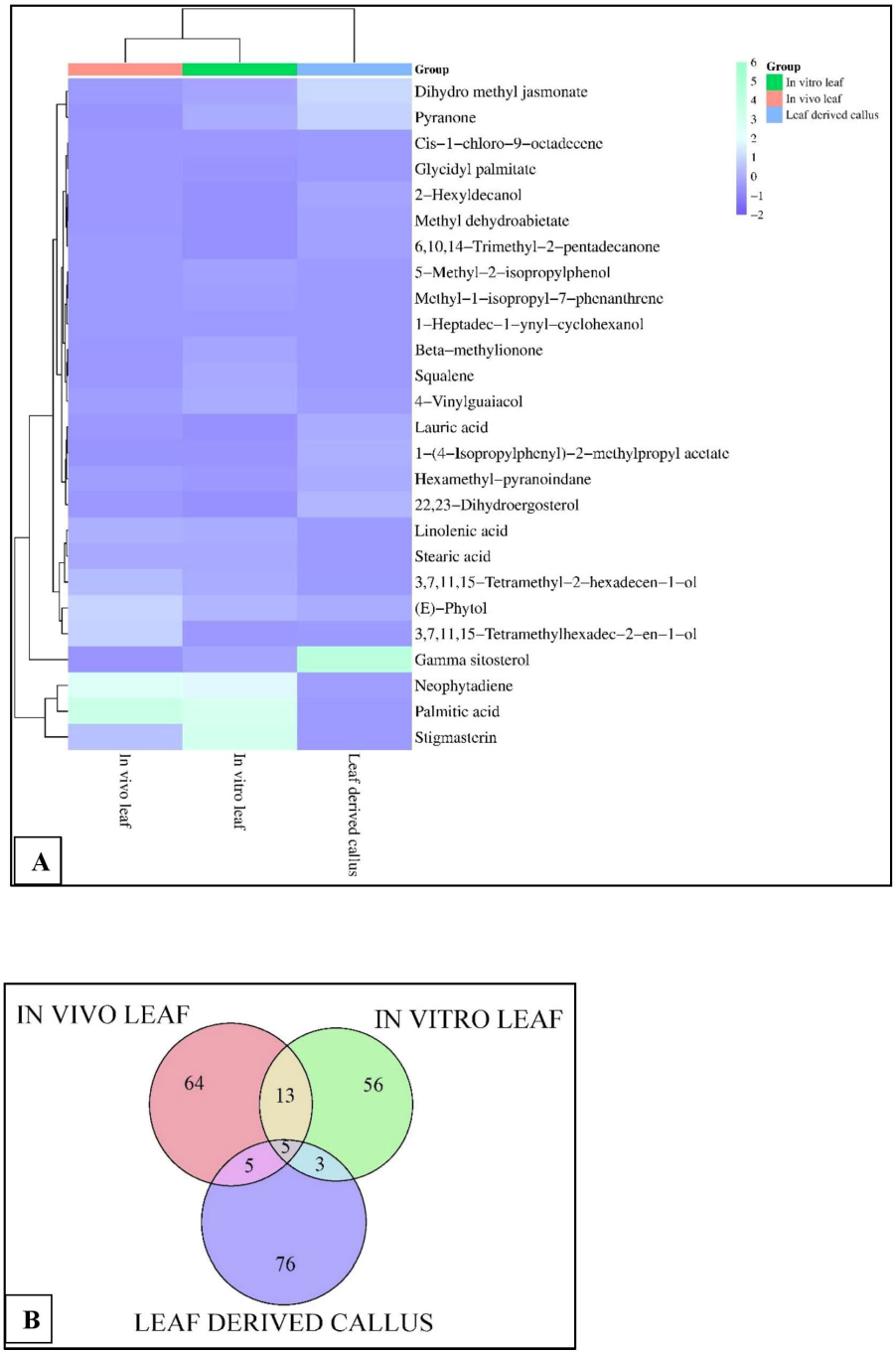

FIGURE 4
(A) Heatmap analysis, and **(B)** Venn diagram displaying the relative abundance of important phytocompounds in the *in vivo*-, *in vitro*- grown leaf and callus samples of *D. purpurea* L. detected by GC-MS.

purpurea were successfully used for callus induction and subsequent shoot regeneration (organogenesis) was achieved by amending MS medium with two most frequently used PGRs i.e. BAP and 2,4-D at varying concentrations. It was observed that a high BAP (8.8 µM) and low 2,4-D (0.9 µM) concentration exerted the best stimulatory effect on callus biomass production. Similar responses have earlier been reported in *D. purpurea* and several other related species (Verma et al., 2016b; Rad et al., 2021). On further sub-culturing, the callus became organogenic and produced green shoots on the same medium. Similarly, a relative higher concentration of BAP (compared to 2,4-D) was found to trigger organogenesis in various plant species such as *Pancratium maritimum* (Yasemin et al., 2023), *P. angulata and P. chenopodifolia* (Romo-Paz et al., 2023). On the contrary, several reports are available where promotive effects of BAP and NAA were noted for shoot organogenesis (de Oliveira et al., 2022; Raju et al., 2022; Liu et al., 2023).

Various internal and external factors like genetic makeup, used agronomic techniques, environmental conditions and *in vitro*

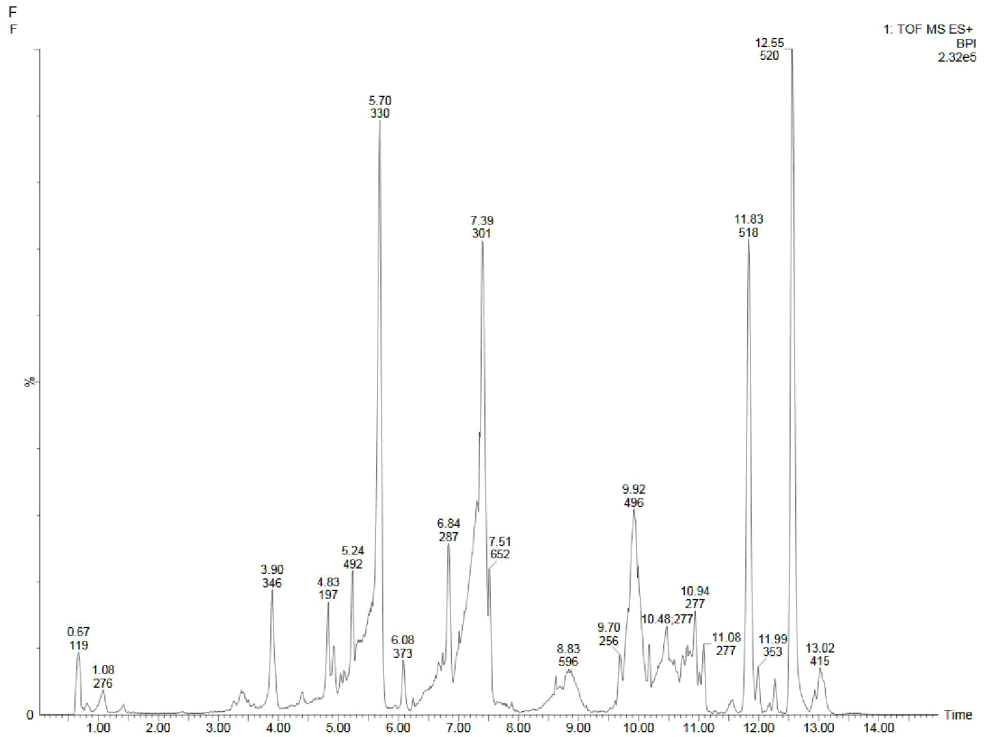

FIGURE 5
UPLC-ESI-QTOF-MS chromatogram of methanolic leaf extract of the *in vivo* grown *D. purpurea* L. in positive ionization mode.

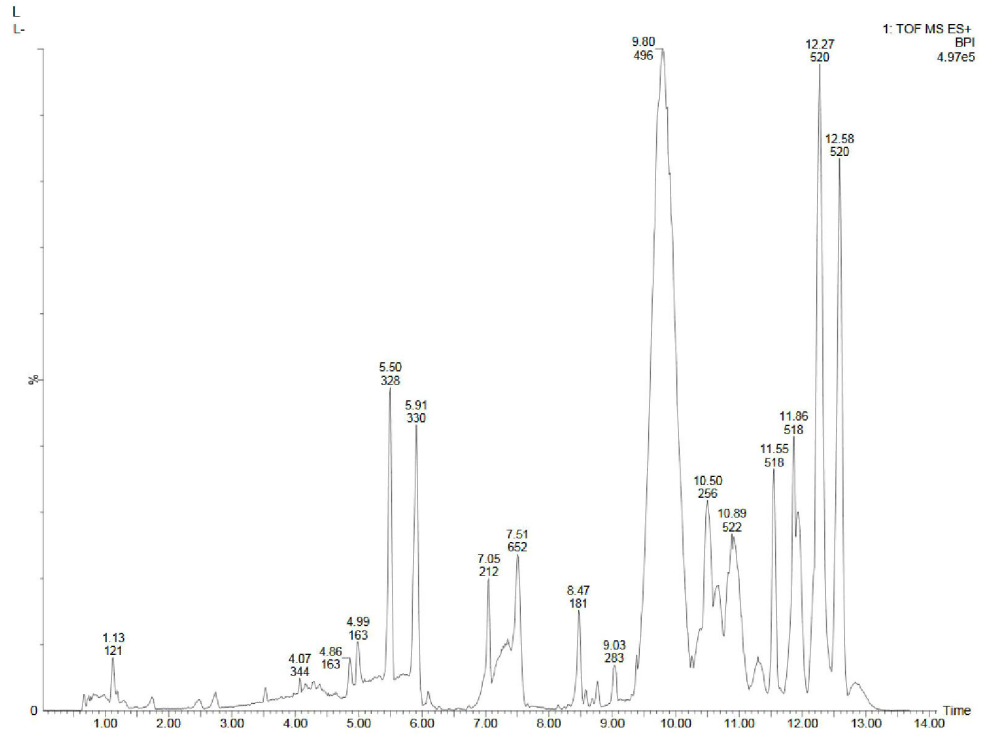

FIGURE 6
UPLC-ESI-QTOF-MS chromatogram of methanolic leaf extract of the *in vitro* grown *D. purpurea* L. in positive ionization mode.

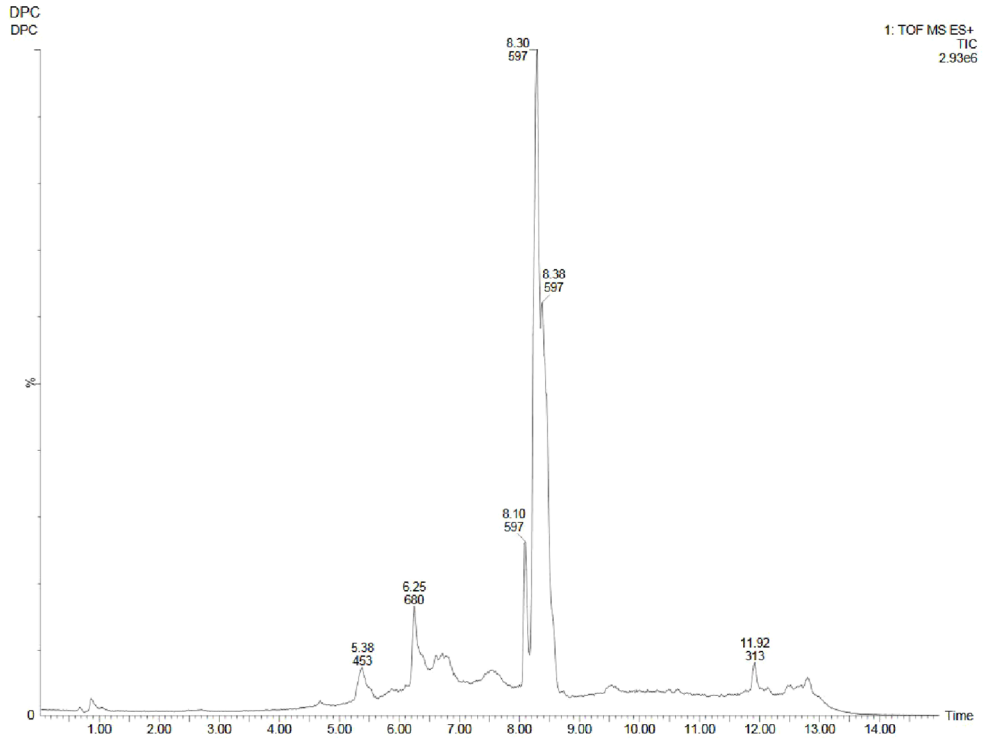

FIGURE 7
UPLC-ESI-QTOF-MS chromatogram of methanolic callus extract of *D. purpurea* L. in positive ionization mode.

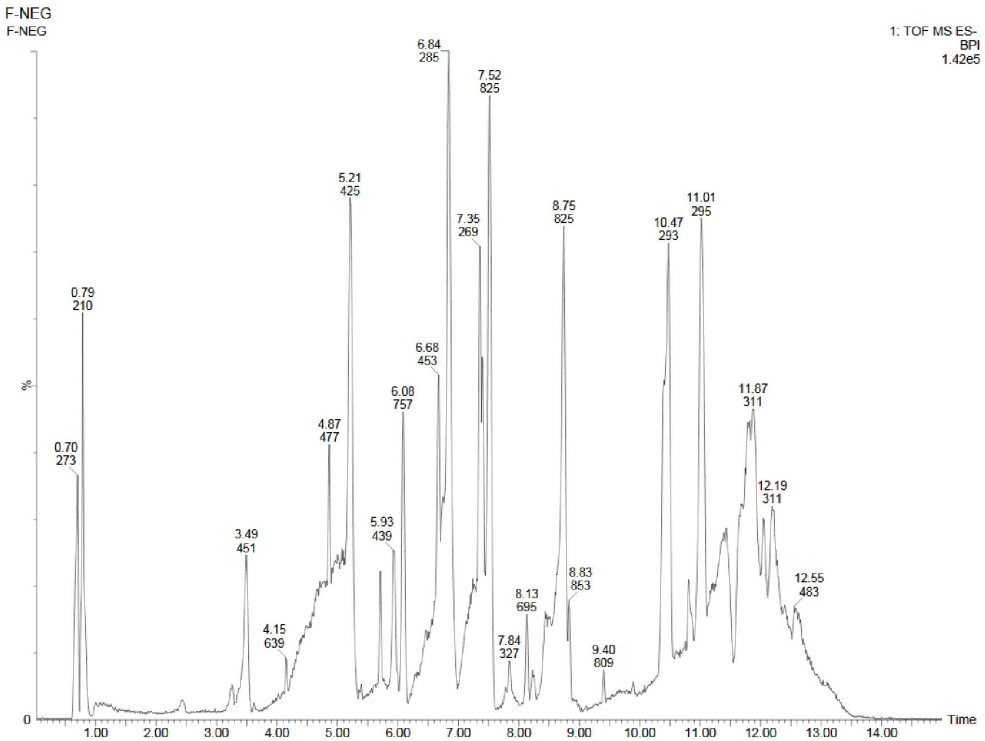

FIGURE 8
UPLC-ESI-QTOF-MS chromatogram of methanolic leaf extract of the *in vivo* grown *D. purpurea* L. in negative ionization mode.

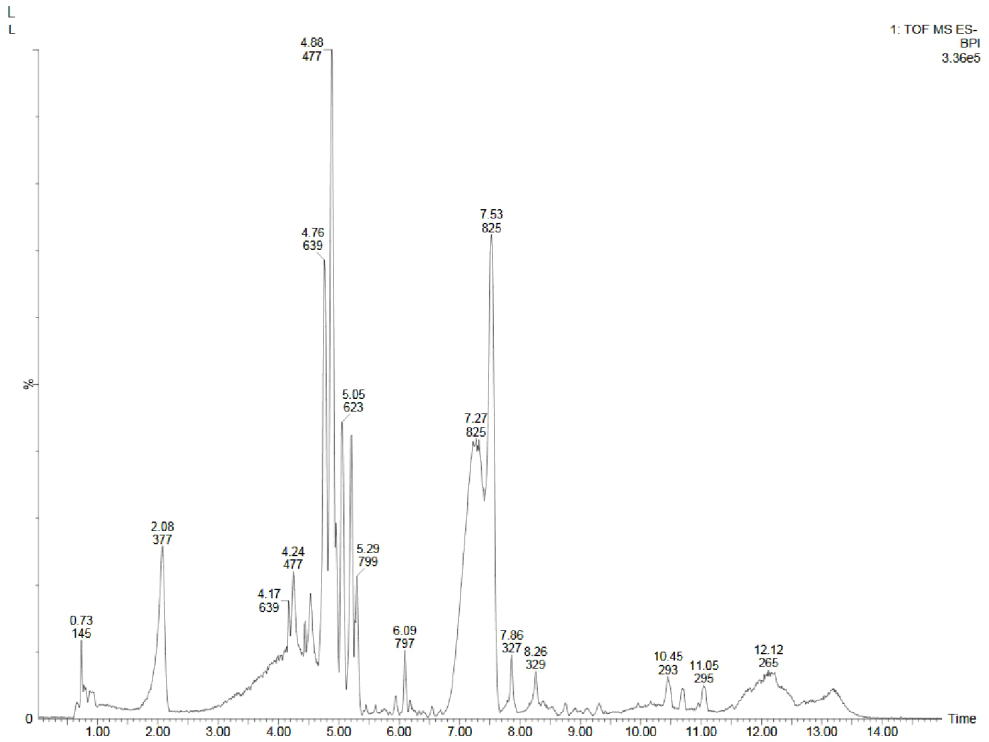

FIGURE 9
UPLC-ESI-QTOF-MS chromatogram of methanolic leaf extract of the *in vitro* grown *D. purpurea* L. in negative ionization mode.

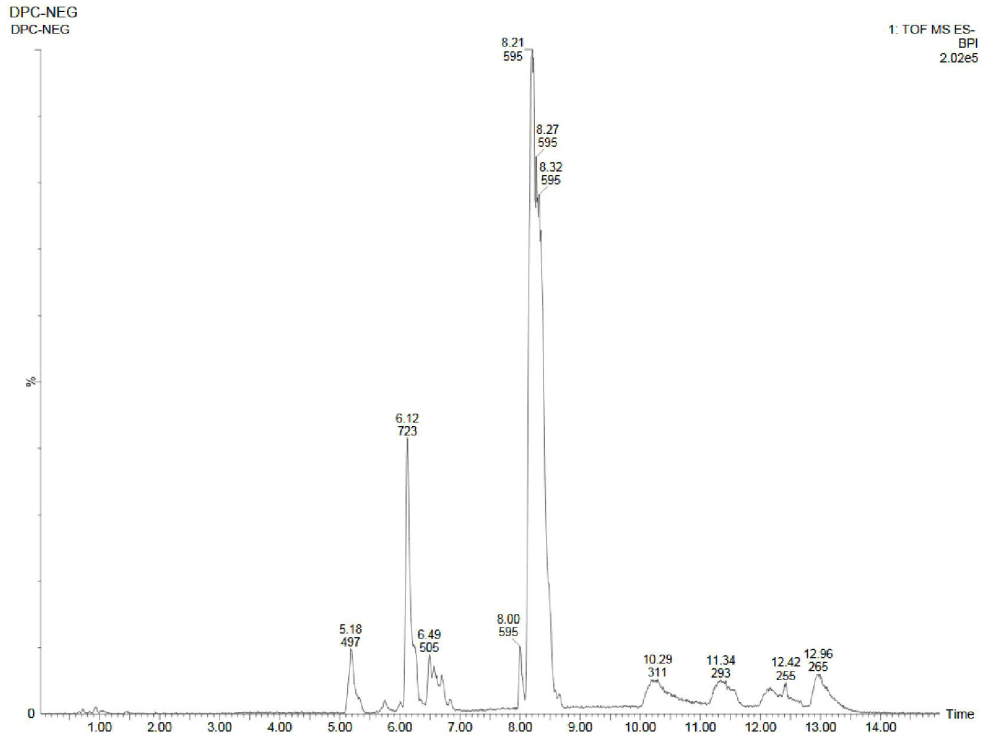

FIGURE 10
UPLC-ESI-QTOF-MS chromatogram of methanolic callus extract of *D. purpurea* L. in negative ionization mode.

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TABLE 9 Identification of phytocompounds in the methanolic leaf extract of *in vivo* grown *D. purpurea* L. by UPLC-ESI-QTOF-MS (positive ion mode) analysis.

S.No.	Rt (min)	Measured m/z	Exact m/z	Name of the compound	Ion	Formula	Product ions (m/z)	Category
1	0.67	119	118.9975	Mesoxalic acid	M+H	$C_3H_2O_5$	19.01, 31.02,44.99,56.99,75.01	Carboxylic acids
2	1.08	276	275.2375	Methyl linolenate	M +H-H2O	$C_{19}H_{32}O_2$	67.05, 79.05, 81.06, 93.06	Fatty acyls
3	3.9	346	346.3104	2,4,12-Octadecatrienoic acidpiperidide	M+H	$C_{23}H_{39}NO$	55.05, 67.05, 69.06, 79.05, 84.08, 86.09, 91.05	Piperidines
4	4.83	197	197.0057	Dehydroascorbic acid	M+Na	$C_6H_6O_6$	43.02, 61.03, 115, 139,157.01, 175.02	Lactones
5	5.24	492	491.3859	Lupeol acetate	M+Na	$C_{32}H_{52}O_2$	409.38, 427.39, 469.40	Triterpenoids
6	5.7	330	330.073	Glutathione	M+Na	$C_{10}H_{17}N_3O_6S$	76.05, 84.08, 129.99	Peptides
7	6.08	373	372.9989	Apigenin-7-sulfate	M+Na	$C_{15}H_{10}O_8S$	119.05, 123.04, 197.06, 232.97, 234.99, 351.01	Flavonoids
8	6.84	287	287.055	Luteolin	M+H	$C_{15}H_{10}O_6$	68.99, 89.03, 117.03,135.04,161.02, 241.04, 287.05	Flavonoids
9	7.39	301	301.0707	Kaempferid	M+H	$C_{16}H_{12}O_6$	283.06, 285.03, 301.07	Flavonoids
10	7.51	652	651.4959	Diglyceride	M+Na	$C_{40}H_{68}O_5$	261.22, 277.25, 335.25, 353.26, 611.50	Diacylglycerol
11	8.83	596	596.1524	Cyanidin 3-galactoside p-coumaric acid ester	M+H	$C_{30}H_{27}O_{13}$	585.32, 596.15, 603.33	Flavonoids
12	9.7	256	257.2475	Octyl octanoate	M+H	$C_{16}H_{32}O_2$	43.05, 55.05, 57.06, 95.08, 127.11	Fatty acyls
13	9.92	496	495.3809	alpha-Tocopherol acetate	M+Na	$C_{31}H_{52}O_3$	57.06, 71.08, 147.08, 165.09, 207.10, 413.37	Vitamin E
14	10.48	277	277.0319	6-Phosphogluconic acid	M+H	$C_6H_{13}O_{10}P$	43.01, 56.99, 59.01,61.02, 73.02, 75.01, 98.98, 105.01, 161.04	Monosaccharide phosphates
15	10.94	277	275.1981	Linalyl hexanoate	M+Na	$C_{16}H_{28}O_2$	41.03, 43.05, 53.03, 55.05, 57.06, 67.05, 69.06, 99.08	Monoterpenoid
16	11.08	277	278.2454	Hexadecanamide	M+Na	$C_{16}H_{33}NO$	211.24, 221.22, 238.25, 239.23, 256.26	Fatty acyls
17	11.83	518	517.1341	Dicaffeoylquinic acid	M+H	$C_{25}H_{24}O_{12}$	117.02, 120.98,144.38, 163.03	Quinic acids and derivatives
18	11.99	353	353.4142	Isopentacosane	M+H	$C_{25}H_{52}$	43.05, 57.07, 225.25, 239.27, 253.28, 295.33	Saturated hydrocarbon
19	12.55	520	521.3109	Digoxigenin monodigitoxoside	M+H	$C_{29}H_{44}O_8$	137.07, 373.23, 391.24, 503.30	Steroid saponin
20	13.02	415	415.2455	20, 22-Dihydrodigoxigenin	M+Na	$C_{23}H_{36}O_5$	357.24, 375.25, 393.26	Cardenolides

culture practices often alter biochemical attributes in the wilds as well as *in vitro* raised plants (Phuyal et al., 2020).Thus, The biochemical and antioxidant markers levels were checked between mother (donor) plant and different *in vitro* raised tissues in *D. purpurea*. From biochemical assays, it was clearly evident that the *in vitro* grown tissues (both callus and organogenic derived shoot leaf) accumulated more phenolics and flavonoids than the donor *D. purpurea* plant. Higher content of phenols and flavonoids in the micropropagated tissues (compared to field grown plant) have also been reported in *Scrophularia takesimensis* (Jeong and Sivanesan, 2015) and *Dendrobium nobile* (Bhattacharyya et al., 2016). As PGRs utilized in *in vitro* culture system, regulate biosynthesis pathway genes, the plant tissue culture system plays a significant role in the production of phenols and flavonoids (Ghosh et al., 2018). The role of clonal propagation is receiving a lot of attention since the primary significance of medicinal plants as a natural source of antioxidants is realized (Bose et al., 2015). Plant tissue, rich in phenols and flavonoids is considered to be a good source of antioxidants as there is a positive correlation of phenolic, flavonoids with antioxidant activities (Khorasani Esmaeili et al., 2015). The antioxidant potential of mother tissues/plants and laboratory grown plants was evaluated by conducting DPPH, POD and SOD assays. DPPH is an easy, reliable and popular method for assessing the ability of plant extracts to scavenge radicals by quenching the stable purple colored DPPH radical into yellow colored DPPH (Aryal et al., 2019). SOD scavenges superoxide radical and manages the levels of H_2O_2, while POD helps in oxidation and decomposition of H_2O_2 (Bansal et al., 2024).

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TABLE 10 Identification of phytocompounds in the methanolic leaf extract of *in vitro* grown *D. purpurea* L. by UPLC-ESI-QTOF-MS (positive ion mode) analysis.

S.No.	Rt (min)	Measured m/z	Exact m/z	Name of the compound	Ion	Formula	Product ions (m/z)	Category
1	1.13	121	121.0624	Hexenal	M+Na	$C_6H_{10}O$	81.07, 99.08	Carbonyl compound
2	4.07	344	343.0818	Rosmarinic acid	M +H-H2O	$C_{18}H_{16}O_8$	123.04, 135.04, 163.03, 181.05, 199.306, 43.08	Polyphenol
3	4.86	163	163.0607	D-Mannose	M +H-H2O	$C_6H_{12}O_6$	43.01, 45.03, 57.03, 61.02, 75.04, 85.02, 103.03, 181.07	Carbohydrates
4	4.99	163	163.1851	(Z)-1,5-Tridecadiene	M +H-H2O	$C_{13}H_{24}$	181.19	Unsaturated hydrocarbon
5	5.5	328	329.3414	1,21-Heneicosanediol	M+H	$C_{21}H_{44}O_2$	311.33, 329.34	Fatty acyls
6	5.91	330	330.073	Glutathione	M+Na	$C_{10}H_{17}N_3O_6S$	76.05, 84.08, 129.99	Peptides
7	7.05	212	213.1485	Dihydrojasmonic acid	M+H	$C_{12}H_{20}O_3$	41.03, 43.01, 55.01, 79.05, 81.06, 83.08	Fatty acyls
8	7.51	652	651.4959	Diglyceride	M+Na	$C_{40}H_{68}O_5$	261.22, 277.25, 335.25, 353.26, 611.50	Diacylglycerol
9	8.47	181	181.1199	2-Heptyl acetate	M+Na	$C_9H_{18}O_2$	41.03, 43.05, 55.05, 57.06, 69.06, 71.08	Carboxylic acids
10	9.03	283	283.0607	Luteolin 7-methyl ether	M +H-H2O	$C_{16}H_{12}O_6$	301.07	Flavonoids
11	9.8	496	495.3809	alpha-Tocopherol acetate	M+Na	$C_{31}H_{52}O_3$	57.06, 71.08, 147.08, 165.09, 207.10, 413.37	Vitamin E compound
12	10.5	256	257.2475	Octyl octanoate	M+H	$C_{16}H_{32}O_2$	43.05, 55.05, 57.06, 95.08, 127.11	Fatty acyls
13	10.89	522	521.5656	5-Hexatriacontanone	M+H	$C_{36}H_{72}O$	503.55	Carbonyl compound
14	11.55	518	517.1341	Dicaffeoylquinic acid	M+H	$C_{25}H_{24}O_{12}$	117.02, 120.98,144.38, 163.03	Quinic acids and derivatives
15	11.86	518	519.0928	Sagecoumarin	M +H-H2O	$C_{27}H_{20}O_{12}$	123.04, 181.04, 311.05, 339.04	Coumarins and derivatives
16	12.27	520	521.3109	Digoxigenin monodigitoxoside	M+H	$C_{29}H_{44}O_8$	137.07, 373.23, 391.24, 503.30	steroid saponin
17	12.58	520	521.0902	Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside	M+Na	$C_{21}H_{22}O_{14}$	153.01, 329.08, 373.07, 443.11, 499.10	Tannins

In our study, it was observed that the laboratory grown tissues (callus and *in vitro* leaf) possessed higher antioxidative activity than the field grown plant parts in all the three assays performed. These findings indicate a much greater antioxidant activity in micropropagated plant tissues compared to the mother plant, consistent with previous experimental observations from other researchers (Zayova et al., 2016; Ali et al., 2018; Mamgain et al., 2023).

Large-scale metabolite identification and/or quantification from one or more samples is referred to as untargeted metabolomic study. The metabolite profile strategy, otherwise called as top-down approach, examines the whole metabolomic profile of a given complicated sample rather than the requirement of a previous comprehensive hypothesis on a particular set of metabolites (de Souza et al., 2022). These can be achieved by performing untargeted GC-MS and LC-MS based metabolite profiling. The GC-MS technique has been applied in several plant species like *Tanacetum sinaicum* (Adel et al., 2021) and *Catharanthus roseus* (Bansal et al., 2023). Till date, there has been no previous untargeted metabolomics investigations in *D. purpurea* that examined *in vitro* regenerated callus and leaf tissue using GC-MS and LC-MS combined approach. In the present study, a comparative metabolite profile of *in vivo-*, *in vitro* grown leaf and leaf-callus has been made by using GC-MS and LC-MS techniques. The methanolic extracts of studied samples revealed the presence of more than 75 phytoconstituents belonging to various classes, like alkaloids, phytosterols, terpenoids, steroids, phenols, sugars etc. These detected bioactive compounds confer this plant diverse

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TABLE 11 Identification of phytocompounds in the methanolic callus extract of *D. purpurea* L. by UPLC-ESI-QTOF-MS (positive ion mode) analysis.

S.No.	Rt (min)	Measured m/z	Exact m/z	Name of the compound	Ion	Formula	Product ions (m/z)	Category
1	5.38	453	453.1391	Epicatechin 3-glucoside	M+H	$C_{21}H_{24}O_{11}$	145.04, 291.08, 393.11, 435.12, 453.13	Flavonoids
2	6.25	680	681.3487	(15a,20R)-Dihydroxypregn-4-en-3-one 20-[glucosyl-(1->4)-6-acetyl-glucoside]	M +H-H2O	$C_{35}H_{54}O_{14}$	315.23, 333.24, 519.29, 537.30	Steroids
3	8.1	597	597.145	Quercetin 3-glucoside 7-xyloside	M+H	$C_{26}H_{28}O_{16}$	435.09, 597.14	Flavonoids
4	8.3	597	597.4126	stigmasterol 3-O-beta-D-glucoside	M+Na	$C_{35}H_{58}O_6$	95.08, 97.10, 395.36, 413.37	Steroids
5	8.38	597	597.5241	Erythrodiol 3-decanoate	M+H	$C_{40}H_{68}O_3$	275.23, 429.37, 443.38, 485.39	Triterpenoids
6	11.92	313	313.1071	3',4',5'-Trimethoxyflavone	M+H	$C_{18}H_{16}O_5$	313.1	Flavonoids

TABLE 12 Identification of phytocompounds in the methanolic leaf extract of *in vivo* grown *D. purpurea* L. by UPLC-ESI-QTOF-MS (negative ion mode) analysis.

S.No.	Rt (min)	Measured m/z	Exact m/z	Name of the compound	Ion	Formula	Product ions (m/z)	Category
1	0.7	273	273.0768	3,3',4',7-Tetrahydroxyflavan	M-H	$C_{15}H_{14}O_5$	121.02, 151.03, 273.07	Flavonoids
2	0.79	210	210.1267	(9z)-12-oxo-dodec-9-enoate	M-H	$C_{12}H_{19}O_3$	167.14, 183.13, 193.12, 211.13	Fatty acyls
3	3.49	451	451.1246	Epicatechin 3-glucoside	M-H	$C_{21}H_{24}O_{11}$	43.01, 109.02, 125.02, 289.07	Flavonoids
4	4.15	639	639.0992	Quercetin 3-(2-caffeoylglucuronoside)	M-H	$C_{30}H_{24}O_{16}$	161.02, 179.03, 283.02, 301.03	Flavonoids
5	4.87	477	477.1039	Nepitrin	M-H	$C_{22}H_{22}O_{12}$	299.01, 315.05	Flavonoids
6	5.21	425	425.3789	alpha-Amyrin	M-H	$C_{30}H_{50}O$	407.36, 425.37	Triterpenoids
7	5.93	439	439.3945	24-Methylcycloartenol	M-H	$C_{31}H_{52}O$	421.38, 439.39	Steroids
8	6.08	757	755.204	Kaempferol 3-coumaroyl-triglucoside	M-H	$C_{33}H_{40}O_{20}$	431.09, 755.20	Flavonoids
9	6.68	453	454.3458	4alpha-carboxy-stigmasta-7,24(241)-dien-3beta-ol	M-H	$C_{30}H_{40}O_3$	393.35, 411.36, 437.34	Steroids
10	6.84	285	285.0405	Luteolin	M-H	$C_{15}H_{10}O_6$	285.03	Flavonoids
11	7.35	269	269.0455	Apigenin	M-H	$C_{15}H_{10}O_5$	269.04	Flavonoids
12	7.52	825	825.6978	Triacylglycerol	M-H	$C_{53}H_{94}O_6$	61.02, 253.21, 279.23, 319.26, 335.25	Triacylglycerol
13	7.84	327	327.3269	1,21-Heneicosanediol	M-H	$C_{21}H_{44}O_2$	297.31, 309.31, 327.32	Fatty acyls
14	8.13	695	697.3441	(15a,20R)-Dihydroxypregn-4-en-3-one 20-[glucosyl-(1->4)-6-acetyl-glucoside]	M-H	$C_{35}H_{54}O_{14}$	59.01, 313.21, 331.22	Steroids
15	8.75	825	827.1888	Luteolin 7-O-(2-apiosyl-4-glucosyl-6-malonyl)-glucoside	M-H	$C_{35}H_{40}O_{23}$	59.01, 84.99, 103, 149.04, 285.03	Flavonoids
16	8.83	853	853.2785	Hydroxymethylbilane	M-H	$C_{40}H_{46}N_4O_{17}$	791.24, 835.26	Tetrapyrroles
17	9.4	809	809.3026	isoorientin 6-O-hexoside	M-H	$C_{42}H_{50}O_{16}$	181.05, 337.12, 415.13, 541.20, 597.19, 641.22, 763.26	lignans
18	10.47	293	293.2122	2-Hydroxylinolenic acid	M-H	$C_{18}H_{30}O_3$	293.21	Fatty acyls
19	11.01	295	295.2643	Nonadecenoic acid	M-H	$C_{19}H_{36}O_2$	277.25, 295.26	Fatty acyls

(Continued)

TABLE 12 Continued

S.No.	Rt (min)	Measured m/z	Exact m/z	Name of the compound	Ion	Formula	Product ions (m/z)	Category
20	11.87	311	311.2956	Ethyl stearate	M-H	$C_{20}H_{40}O_2$	265.25	Fatty acyls
21	12.19	311	311.2017	Delta4,16-Pregnadiene-3,20-dione	M-H	$C_{21}H_{28}O_2$	269.19, 295.17	Steroids
22	12.55	483	483.078	2,6-Digalloylglucose	M-H	$C_{20}H_{20}O_{14}$	125.02, 169.01	Benzenoids

therapeutic importance. The major metabolites found in methanolic leaf extract of *in vivo* plant were 4-vinylguaiacol, lauric acid, myristic acid, loliolide, palmitic acid, squalene, stigmasterin, d-alpha-tocopherol etc. Loliolide is a type of monoterpenoid hydroxylactones, exhibiting anti-proliferative, anti-bacterial, anti-fungal and allelochemic activities and is reported in various plant species such as *Rauvolfia yunnanensis*,

Veronica persica, *Salvia divinorum* etc (Grabarczyk et al., 2015). Stigmasterin (or Stigmasterol) shows diverse range of pharmacological effects like anti-diabetic, immunomodulatory, antiparasitic, anticancer, anti-osteoarthritis, anti-inflammatory, antifungal properties (Bakrim et al., 2022). Squalene, a triterpenoid, possess antioxidant, anti-inflammatory, anti-neoplastic and anti-atherosclerotic properties (Lou-Bonafonte et al., 2018).

Similarly, the methanolic leaf extract of *in vitro* raised plant had 77 phytocompounds such as neophytadiene, phytol, beta-methylionone, linolenic acid, stearic acid, campesterol, gamma sitosterol, cycloartenol etc. Neophytadiene and phytol are diterpenoid compounds with anti-microbial, anti-inflammatory, anti-cancerous and antipyretic activities, which have been detected by GC-MS in various plant species (Willie et al., 2020; Banni and Jayaraj, 2022). Beta-sitosterol is widely known for its anti-diabetic, anti-inflammatory, cytotoxic and immunosuppressive properties (Salazar et al., 2020), campesterol (another sterol) has been associated with cancer prevention, anti-fungal and cholesterol lowering activities (Uttu et al., 2023). Callus extract on the other hand, showed a more varied range of metabolites such as linalool acetate, eugenol, cinnamic acid, jasmone, alpha-monostearin, 3-hydroxy-5-methoxyflavone, beta-amyrin, cycloartenol etc. in varied

TABLE 13 Identification of phytocompounds in the methanolic leaf extract of *in vitro* grown *D. purpurea* L. by UPLC-ESI-QTOF-MS (negative ion mode) analysis.

S.No.	Rt (min)	Measured m/z	Exact m/z	Name of the compound	Ion	Formula	Product ions (m/z)	Category
1	0.73	145	145.0295	Coumarin	M-H	$C_9H_6O_2$	101.03, 145.02	Coumarins and derivatives
2	2.08	377	376.0378	2-Hydroxypropyl glucosinolate	M-H	$C_{10}H_{19}NO_{10}S_2$	111.97, 195.97, 376.03	Carbohydrates
3	4.17	639	639.0992	Quercetin 3-(2-caffeoylglucuronoside)	M-H	$C_{30}H_{24}O_{16}$	161.02, 179.03, 283.02, 301.03	Flavonoids
4	4.24	477	477.1039	Nepitrin	M-H	$C_{22}H_{22}O_{12}$	299.01, 315.05	Flavonoids
5	4.76	639	639.1567	Rhamnetin 3-laminaribioside	M-H	$C_{28}H_{32}O_{17}$	315.05, 639.15	Carboxylic acids
6	4.88	477	477.1402	4'-O-Methyl-(-)-epicatechin-5-O-β-glucuronide	M-H	$C_{23}H_{26}O_{11}$	163.07, 283.09, 301.10	Flavonoids
7	5.05	623	623.1618	3,8-Diglucosyldiosmetin	M-H	$C_{28}H_{32}O_{16}$	623.16	Flavonoids
8	5.29	799	799.6457	Campesterol 6'-hexadecanoylglucoside	M-H	$C_{50}H_{88}O_7$	399.36, 543.40, 561.41, 799.64	Steroids
9	6.09	797	797.1418	Apigenin 7-[glucuronyl-(1->2)-glucuronide] 4'-glucuronide	M-H	$C_{33}H_{34}O_{23}$	444.06	Flavonoids
10	7.27	825	827.1888	Luteolin 7-O-(2-apiosyl-4-glucosyl-6-malonyl)-glucoside	M-H	$C_{35}H_{40}O_{23}$	59.01, 84.99, 103, 149.04, 285.03	Flavonoids
11	7.53	825	825.6978	Triacylglycerol	M-H	$C_{53}H_{94}O_6$	61.02, 253.21, 279.23, 319.26, 335.25	Triacylglycerol
12	7.86	327	327.3269	1,21-Heneicosanediol	M-H	$C_{21}H_{44}O_2$	297.31, 309.31, 327.32	Fatty acyls
13	8.26	329	329.2122	pregn-5-ene-3,20-dione-17-ol	M-H	$C_{21}H_{30}O_3$	287.20, 329.21	Steroids
14	10.45	293	293.2122	2-Hydroxylinolenic acid	M-H	$C_{18}H_{30}O_3$	293.21	Fatty acyls
15	11.05	295	295.2643	Nonadecenoic acid	M-H	$C_{19}H_{36}O_2$	277.25, 295.26	Fatty acyls
16	12.12	265	264.952	2,3-Diphosphoglyceric acid	M-H	$C_3H_8O_{10}P_2$	78.95, 96.96	Carbohydrates

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TABLE 14 Identification of phytocompounds in the methanolic callus extract of *D. purpurea* L. by UPLC-ESI-QTOF-MS (negative ion mode) analysis.

S.No.	Rt (min)	Measured m/z	Exact m/z	Name of the compound	Ion	Formula	Product ions (m/z)	Category
1	5.18	497	497.0937	Methyl 4,6-di-O-galloyl-β-D-glucopyranoside	M-H	$C_{21}H_{22}O_{14}$	125.02, 169.01, 441.10	Tannins
2	6.12	723	723.1719	2",3"-Di-O-p-coumaroylafzelin	M-H	$C_{39}H_{32}O_{14}$	151.00, 723.17	Flavonoids
3	6.49	505	505.0988	Quercetin 3-(6"-acetylglucoside)	M-H	$C_{23}H_{22}O_{13}$	59.01, 301.03	Flavonoids
4	8	595	595.4579	Squamoxinone C	M-H	$C_{35}H_{64}O_{7}$	577.44, 595.45	Fatty acyl
5	8.21	595	595.5096	Erythrodiol 3-decanoate	M-H	$C_{40}H_{68}O_{3}$	595.5	Triterpenoids
6	8.27	595	595.1668	Cassiaside C	M-H	$C_{27}H_{32}O_{15}$	59.01, 241.05, 271.06	Naphthopyrans
7	8.32	595	595.4215	Diacylglycerol	M-H	$C_{34}H_{60}O_{8}$	195.17, 213.18, 307.19, 381.22, 451.30	Diacylglycerol
8	10.29	311	311.2017	Delta4,16-Pregnadiene-3,20-dione	M-H	$C_{21}H_{28}O_{2}$	269.19, 295.17	Steroids
9	11.34	293	293.2122	2-Hydroxylinolenic acid	M-H	$C_{18}H_{30}O_{3}$	293.21	Fatty acyl
10	12.42	255	255.233	Octyl octanoate	M-H	$C_{16}H_{32}O_{2}$	125.09, 143.10, 255.23	Fatty acyl
11	12.96	265	264.952	2,3-Diphosphoglyceric acid	M-H	$C_{3}H_{8}O_{10}P_{2}$	78.95, 96.96	Carbohydrates

quantities. Eugenol (volatile phenolic compound) has been detected in several plant species, namely *Eugenia caryophyllata*, *Myristica fragrans*, *Ocimum basilicum* and act as antifungal, analgesic, anticancer, antiparasitic, antioxidant and antimicrobial agent (Abdou et al., 2021). Cinnamic acid is a key compound of aromatic carboxylic acid group, possessing anti-diabetic, anti-inflammatory, anti-cancerous and anti-microbial activity and is widely found in a number of plant varieties like *Cinnamomum cassia*, *Panax ginseng* etc (Ruwizhi and Aderibigbe, 2020). Beta-amyrin (a triterpenoid) demonstrates analgesic, anti-inflammatory, gastroprotective, hepatoprotective, anticonvulsant, antidepressive, antipancreatitic effects (Nogueira et al., 2018). This differential presence of metabolites in *in vitro* regenerated tissues (as compared to the mother plant parts) may be attributed to specific ecotype, genotype, explant, relative humidity, photoperiod, temperature, PGRs exposure and other variables in cultured conditions (Khan A. et al., 2021).

Finally, the methanolic extracts of each sample were subject to LC-MS analyses and the observation revealed that the *Digitalis purpurea* is enriched with a wide variety of phytocompounds such as flavonoids, fatty acyls, terpenoids, saponins, cardenolides, sugars, steroids, tannins and lignans, thereby increases plant's medicinal potential. Several detected compounds in this present work, were reported previously in other Plantaginaceae members. These are like apigenin (269.04 m/z), Dicaffeoylquinic acid (517.13 m/z), luteolin (285.04 m/z), Quercetin 3-(2-caffeoylglucuronoside) (639.09 m/z) etc (Nedime et al., 2023; Bouali et al., 2024). The most significant phytocompound groups identified in both positive and negative ionization modes perhaps were flavonoids, fatty acyl and steroids. Major flavonoids detected in the current study include 3,3',4',7-tetrahydroxyflavan, nepitrin, luteolin, apigenin and kaempferid. Nepitrin has earlier been isolated from *Rosmarinus officinalis* and *Salvia plebeia* (Slimestad et al., 2022), both belonging to the same order Lamiales just like *Digitalis purpurea*. Kaempferid (299 m/z), luteolin (285 m/z) and apigenin (269 m/z) were also identified in the leaf extracts of *Digitalis trojana*. Flavonoids represent the most extensive category of naturally occurring compounds, with over 9000 phenolic chemicals identified in plants (Shomali et al., 2022). The application of flavonoids is quite widespread which includes antitumor, neuroprotective, anticancer, antibacterial, antiviral, antiangiogenic, antioxidant, and anti-proliferative activities (Ullah et al., 2020).

Fatty acyl followed by steroids group was the next most abundant metabolites detected in the present work. The fatty acyl group includes 2-hydroxylinolenic acid, ethyl stearate, octyl octanoate, dihydrojasmonic acid. Dihydrojasmonic acid is well known for its anti-cancerous, anti-depressant, anti-inflammatory, anti-nociceptive, anti-parasitic activities (Ghasemi Pirbalouti et al., 2014). 24-methylcycloartenol, delta4, 16-pregnadiene-3,20-dione, pregn-5-ene-3,20-dione-17-ol, campesterol 6'-hexadecanoylglucoside, stigmasterol 3-o-beta-d-glucoside were some of the major steroidal compounds identified in the tested samples. Campesterol and stigmasterol are the important phytosterols, found to be accumulated in *D. purpurea* tissues and act as precursor sterol for cardenolide biosynthesis (Carroll et al., 2023; Kunert et al., 2023). Two interesting derivatives of digoxigenin (and digoxin metabolite) i.e., digoxigenin monodigitoxoside and 20,22-dihydrodigoxigenin were detected in positive ion modes in *in vivo*- and *in vitro* grown leaf samples of *D. purpurea*. Their presence indicated that the cardenolides biosynthetic pathway was operational in studied experimental leaf tissue of both field grown and laboratory grown *D. purpurea*. Some other important metabolites identified were alpha-amyrin, rosmarinic acid, 2,3-diphosphoglyceric acid. Rosamarinic acid is a polyphenol, exhibiting antioxidant, anti-bacterial, anti-viral and anti-inflammatory activities (Andrade et al., 2018). In general, the callus extract showed the least number of

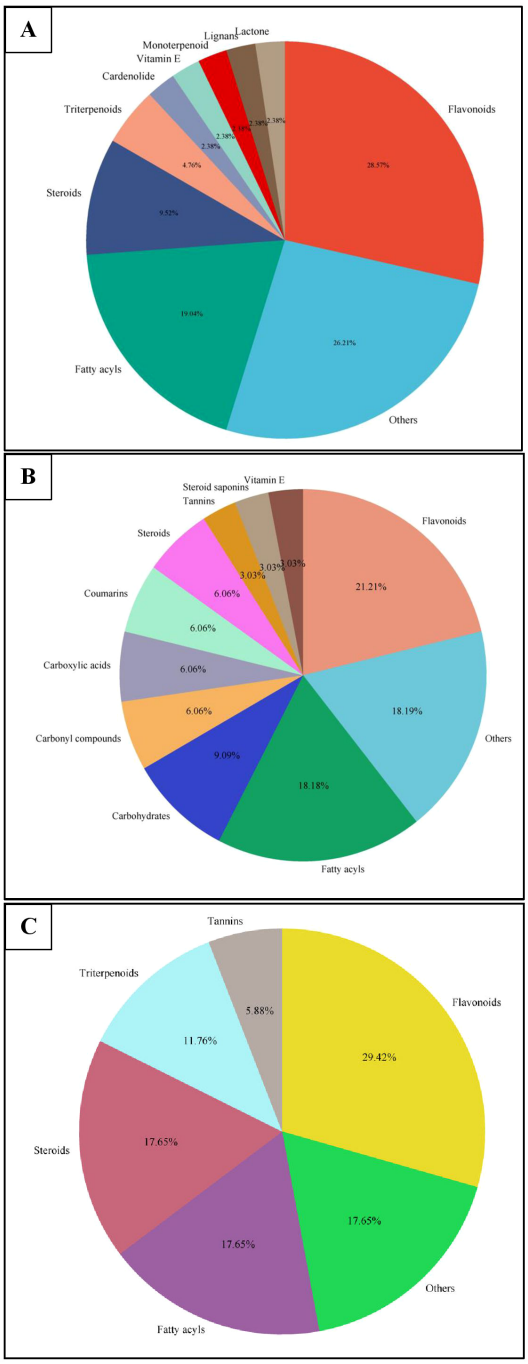

FIGURE 11
Pie-charts showing the proportions of the most abundant categories of phytocompounds detected in UPLC-ESI-QTOF-MS analysis of **(A)** *in vivo* grown leaf tissue, **(B)** *in vitro* grown leaf tissue, and **(C)** leaf derived callus of *D. purpurea* L.

phytocompounds in both positive and negative ionization modes as compared to the *in vivo*- and *in vitro*-grown leaves. This may be due to the fact that the callus is a simple tissue, some degree of cellular differentiation and tissue organization are necessary in controlling synthesis and accumulation of secondary metabolites (Karakas and Turker, 2016). These studies may pave the way for broad-spectrum drug development after ascertaining the bioactivity, toxicity and clinical trials of identified bioactive.

5 Conclusions

A comparative analysis of biochemical parameters, antioxidant activities and metabolite profiles of mother/donor plant and *in vitro* regenerated leaf tissues and leaf-callus were studied. To our knowledge, the current work represents the first metabolic profiling study of *in vitro* cultured tissues in *Digitalis purpurea* by GC-MS and UPLC-ESI-QTOF-MS techniques. The biochemical

and antioxidant attributes showed that the *in vitro* derived tissues (callus and leaf samples) had higher level of phenols, flavonoids as well as antioxidant activities than the field grown (mother) plant. A variety of phytocompounds were identified and quantified in each sample by using GC-MS approach, revealing diverse pharmacological effects of this plant. The phytochemical composition of methanolic extracts of tissues were assayed by using UPLC-ESI-QTOF-MS in positive and negative modes. Major phytoconstituents detected were flavonoids, fatty acyls, steroids, triterpenoids, carbohydrates etc. The variation in metabolites of studied sample may be attributed to a number of factors like explant/tissue specific, genotype, used PGRs concentration, *in vitro* culture conditions etc. These analyses confirmed diverse therapeutic value of *D. purpurea;* the *in vitro* culture may therefore, be exploited for production of important bioactive compounds for pharmaceutical industry. Furthermore, studies like molecular docking and bio-prospecting, could be performed to deduce the ligand-protein interaction and biological properties of these therapeutically important phytocompounds, which will lead to pre-clinical and clinical trials in the later stages.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

YB: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. AM: Conceptualization, Investigation, Supervision, Writing – review & editing. JM: Data curation, Investigation, Methodology, Writing – review & editing. RS: Data curation, Investigation, Project administration, Writing – review & editing. MM: Data curation, Writing – review & editing. AN: Formal analysis, Writing – review & editing. YD: Formal analysis, Methodology, Writing – review & editing. NM-D: Investigation, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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