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RESEARCH ARTICLE

Optimizing callogenesis in five potential medicinal herbs for the bioactive constituents: a sustainable approach to pharmaceutical production

Muhammad Wasim Haider · Muhammad Nafees · Maryyam Bint-e-Tariq · Umar Farooq · Tanveer Hussain · Taki Demir · Asad Masood · Muhammad Samsam Raza · Abd El-Zaher M. A. Mustafa · Humaira Rizwana · Ozhan Simsek · Temoor Ahmed · Atman Adiba · Rashid Iqbal

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Abstract The search for natural antioxidants to safeguard against several diseases is expanding rapidly. Interestingly, the levels of antioxidants have been discovered to be greater in the in vitro-raised calli than the plant extracts in vivo. The aim of this research was to standardize the protocols for culturing calli of five potential medicinal herbs and determine their antioxidant and polyphenolic compounds. The calli of carnation, goji berry, harmal, bitter cucumber, and datura were developed from young leaves using Murashige and Skoog media with varied forms and concentrations of cytokinin and auxin in combination after their optimization. Goji berry, carnation, and datura initiated callus in 13 days, faster than bitter cucumber (20 days). Datura had a 28.7% higher callus induction rate than bitter cucumber. The callus weight of goji berry was three times higher than harmal,

M. W. Haider (✉) · M. Nafees · M. Bint-e-Tariq · U. Farooq · M. S. Raza
Department of Horticultural Sciences, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
e-mail: wasim.haider@iub.edu.pk

M. Nafees
e-mail: muhammad.nafees@iub.edu.pk

M. Bint-e-Tariq
e-mail: maryyamtariq12@gmail.com

U. Farooq
e-mail: umargillhort@gmail.com

M. S. Raza
e-mail: samsam.raza@gmail.com

T. Hussain
Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi 46300, Pakistan
e-mail: dr.tanveer005@uaar.edu.pk

T. Demir
Department of Horticulture, Faculty of Agriculture, Sakarya University of Applied Sciences, Serdivan, Turkey
e-mail: tdemir@subu.edu.tr

A. Masood
Technical Support-Crop Protection Development, Syngenta, Multan 60000, Pakistan
e-mail: asad.masood@syngenta.com

A. E.-Z. M. A. Mustafa · H. Rizwana
Department of Botany and Microbiology, College of Science, King Saud University, P.O. 2455, 11451 Riyadh, Saudi Arabia
e-mail: amus@ksu.edu.sa

H. Rizwana
e-mail: hrizwana@ksu.edu.sa

O. Simsek
Agriculture Faculty, Horticulture Department, Erciyes University, 38030 Kayseri, Turkey
e-mail: ozhan12@gmail.com

T. Ahmed
Advanced Research Centre, European University of Lefke, Lefke, Northern Cyprus, TR-10, Mersin, Turkey
e-mail: temoorahmed248@gmail.com

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with a 25.4% greater diameter than bitter cucumber. The callus of goji berry had 4.3 times more phenolic and ascorbic content than datura and 1.9× more than harmal. The callus of datura had twice the total antioxidant capacity of harmal. The callus of goji berry exhibited 5.7% increased radical-scavenging activities than datura. The enzyme activities of catalase and superoxide dismutase were 2.6% and 2.4% greater in the callus of goji berry than datura. The callus of goji berry also had 2.1% and 2.4% increased peroxidase and ascorbate peroxidase activities than datura and bitter cucumber, respectively. From the findings, it can be concluded that the callus of goji berry is a highly promising source of natural antioxidants, exhibiting significantly higher levels of antioxidant and polyphenolic compounds compared to other medicinal herbs.

Keywords Bitter cucumber · Callus diameter and weight · Callus induction percentage · Carnation · Datura · Enzymatic and non-enzymatic antioxidants · Goji berry · Harmal · Herbal medicine

Abbreviations

2,4-D	2,4-Dichlorophenoxy acetic acid
AAC	Ascorbic acid content
ABTS	2,2-Azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid
ANOVA	Analysis of variance
BAP	Benzyl aminopurine
CD	Callus diameter
CW	Callus weight
DPPH-RSA	2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay
DoHS	Department of Horticultural Sciences
IUB	The Islamia University of Bahawalpur
LSD	Least significant difference
MS media	Murashige and Skoog media
NAA	Naphthalene acetic acid
POD	Peroxidase
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TFC	Total flavonoid content
TPC	Total phenolic content

T. Ahmed
MEU Research Unit, Middle East University, Amman, Jordan

A. Adiba
Regional Agricultural Research Center of Tadla, National Institute of Agricultural Research, Avenue Ennasr, P.O. Box 415, 10090 Rabat, Morocco
e-mail: adiba.atman@gmail.com

R. Iqbal (✉)
Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur Pakistan, Bahawalpur 63100, Pakistan
e-mail: rashid.iqbal@iub.edu.pk

R. Iqbal
Department of Life Sciences, Western Caspian University, Baku, Azerbaijan

Introduction

Herbal therapy is the scientific practice of using plants to treat diseases and disorders (Ahmed et al. 2014; David et al. 2015). In the last few decades, it has become increasingly mainstream with developments and advancements in clinical research as well as quality control (Atanasov et al. 2015). Medicinal herbs encompass a number of plants with therapeutic properties. These plant species provide a paradoxical source of bioactive compounds that can be restored to enhance pharmaceutical advancement (Hossen et al. 2023; Ismail et al. 2023). According to an estimate, the annual value of the global market for herbal plants is around US$62. Moreover, about 50% of all medications are derived from plants that perform a vital role in the pharmaceutical sector (Savithramma et al. 2016; Shakya 2016; Shaukat et al. 2023). Fortunately, Pakistan is bestowed with abundant medicinal herbs of almost 2000 species, including carnation (*Dianthus caryophyllus*), goji berry (*Lycium barbarum*), harmal (*Penganum harmala*), bitter cucumber (*Cucumis callosus*), and datura (*Datura stramonium*) with tremendous therapeutic potential (Ahmed et al. 2004; Bibi et al. 2011; Rehman et al. 2014; Khan et al. 2017; Danquah et al. 2023). They have antibacterial, anti-cancer, anti-diabetic, immune-boosting, skin-protective, cardioprotective, neuroprotective, and anti-inflammatory effects on humans (Ahangarpou et al. 2019; Ciumărnean et al. 2020; Pangaribuan et al. 2023). However, Pakistan's medicinal plant trade revenue is far lower than that of neighboring countries such as India and China (Ullah 2017). This is possibly due to a lack of research on new medicinal herbs or an assessment of existing medicinal herbs for potential bioactive compounds.

Phytocompounds are crucial in traditional remedies (Harakeh et al. 2017; Bharathi et al. 2022; Chaudhary et al. 2023; Rejab et al. 2023). The most commonly used phytochemical molecules in medicines are phenolics, flavonoids, enzymatic antioxidants, such as superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and scavengers of reactive oxygen species (ROS) (Zhang et al. 2015; Tungmunnithum et al. 2018). Nowadays, a number of investigations have discovered that extracts of diverse natural resources, including medicinal plants, hold significant potential for combating numerous ailments (Chaudhuri et al. 2015; Ghate et al. 2016; Panja et al. 2016; Kupradit et al. 2023). Thus, the term "Green Drug" has developed to describe the practice of using plant phytochemicals that have been extracted and refined, either with or without slight chemical alterations (Basu et al. 2017; Sennoi et al. 2023). In the recent era, under controlled conditions, callus culture technology has developed an enormous improvement in the content of bioactive compounds (Ali et al. 2018; Baloch et al. 2024). The calli extracts of medicinal plants are high in these compounds as compared to their in vivo plant extracts (Vignesh et al. 2022; Taratima et al. 2023). The callus culture technique has also enabled the sustainable production of a product throughout the year, irrespective of external climate or soil conditions (Pakseresht et al. 2016; Babich et al. 2020). Past research has documented the callus production protocols of certain medicinal plants, including *Dorem ammoniacum* (Irvani et al. 2010), *Stevia rebaudiana* (Janarthanam et al. 2010; Keshvari et al. 2018), *Tribulus terrestris* (Sharifi et al. 2012), *Celosia argentea* (Bakar et al. 2014), *Achyranthes aspera* (Sen et al. 2014), *Grewia carpinifolia* (Adebiyi et al. 2017), and *Datura inoxia* (Tardast et al. 2023). However, no comprehensive study has been found to develop the callus production protocols of the majority of medicinal herbs for their potential assessment of bioactive compounds of pharmaceutical importance. Therefore, it is vital to extract and identify these bioactive compounds and standardize their protocols in order to ensure their sustainable supply to the pharmaceutical sector.

Keeping in view the above-mentioned questions, this study was set out with two primary objectives: 1. To optimize callus formation in carnation, goji berry, harmal, bitter cucumber, and datura; 2. To assess the bioactive compounds in their calli, particularly enzymatic and non-enzymatic, and total antioxidants, including ROS scavengers.

Materials and methods

Explant collection of medicinal herbs and their sterilization

The young leaves (ageing one week) of five-month-old experimental medicinal herbs, including carnation (*Dianthus caryophyllus*) var. Domingo, goji berry (*Lycium barbarum*) var. Damaye, wild harmal (*Penganum harmala*), wild bitter cucumber (*Cucumis callosus*), and wild datura (*Datura stramonium),* were collected as explants from the Horticulture Experimental Area (29°22′17.4″ N 71°45′53.6″ E), Department of Horticultural Sciences (DoHS), The Islamia University of Bahawalpur (IUB), Pakistan following established protocol and permission was obtained. Our plant study complies with relevant institutional, national and international guidelines and legislation. Further, the plant material was identified and taxonomically validated by well-known horticulturist Dr. Muhammad Wasim Haider, Lecturer at Department of Horticultural Sciences, The Islamia University of Bahawalpur, Pakistan. The voucher specimens were submitted to gene bank of The Islamia University of Bahawalpur, Department of Horticultural Sciences, for allotment of voucher number (HORT-122), multiplication and to ensure availability for future use. The leaves were then shifted to the Plant Tissue Culture Laboratory, DoHS, IUB, within 10 min, where they were surface sterilized by washing with distilled water to eliminate dust particles. Subsequently, they were exposed to a 0.1% $HgCl_2$ solution for 5 min, followed by rinsing with sterile distilled water three times. Finally, the leaves were dried under aseptic conditions (Ahmad et al. 2010). The leaf explants were then excised into discs of 10 mm × 5 mm for inoculation. The leaf discs were cultured on MS media (Murashige and Skoog 1962), supplemented with altered concentrations of plant growth regulators (PGRs).

Preparation of culture media and culture establishment

MS media comprised of 3% sucrose, 0.8% agar, and 0.2% myo-inositol, as well as several macro- and micronutrient salts, including calcium chloride, potassium nitrate, ammonium nitrate, manganese sulfate heptahydrate, magnesium sulfate, potassium phosphate monobasic, zinc sulfate heptahydrate, cupric sulfate, potassium iodide, sodium molybdate, cobalt chloride, and boric acid, and vitamins such as pyridoxine–HCl, thiamine-HCl, glycine, and nicotinic acid, supplemented with different concentrations of selected PGRs. The pH of MS media was maintained to 5.8 ± 0.2 prior to the addition of agar and then autoclaved for 20 min at 121 °C and 15 lb. pressure. The cultures were kept in the maintained temperature of 25 ± 2 °C, relative humidity of 60–70%, and 300 µmol m^{-2} s^{-1} light intensity for 16 h of light and 8 h of darkness. Every treatment was replicated four times.

Optimization of PGRs

Carnation

Callus induction in carnation was carried out by supplementing MS media with 0.5 mg L^{-1} kinetin and 2 mg L^{-1} naphthalene acetic acid (NAA) after optimizing their doses in preliminary assessment, including 0, 0.25, 0.5, 0.75, 1, and 1.25 mg L^{-1} kinetin and 0, 0.5, 1, 1.5, 2, and 2.5 mg L^{-1} NAA.

Goji berry

Callus induction in goji berry was achieved by supplementing MS media with 1 mg L^{-1} benzyl aminopurine (BAP) and 2 mg L^{-1} 2,4-dichlorophenoxy acetic acid (2,4-D) after optimizing their doses, including 0, 0.5, 1, 1.5, 2, and 2.5 mg L^{-1} BAP and 0, 0.5, 1, 1.5, 2, and 2.5 mg L^{-1} 2,4-D.

Harmal

Callus induction in harmal was attained by supplementing MS media with 0.5 mg L^{-1} benzyl aminopurine (BAP) and 1 mg L^{-1} 2,4-dichlorophenoxy acetic acid (2,4-D) after optimizing their doses, including 0, 0.5, 1, 1.5, 2, and 2.5 mg L^{-1} BAP and 0, 0.5, 1, 1.5, 2, and 2.5 mg L^{-1} 2,4-D.

Bitter cucumber

Callus induction in bitter cucumber was performed by supplementing MS media with 2 mg L^{-1} BAP and 0.5 mg L^{-1} NAA after optimizing their doses, including 0, 0.5, 1, 1.5, 2, and 2.5 mg L^{-1} BAP and 0, 0.25, 0.5, 0.75, 1, and 1.25 mg L^{-1} NAA.

Datura

The callus formation in datura was carried out by adding 1 mg L^{-1} BAP and 2 mg L^{-1} 2,4-D to MS media, after optimizing their doses including 0, 0.25, 0.5, 0.75, 1, and 1. 25 mg L^{-1} for BAP, and 0, 0.5, 1, 1.5, 2, and 2. 5 mg L^{-1} for 2,4-D.

Studied attributes

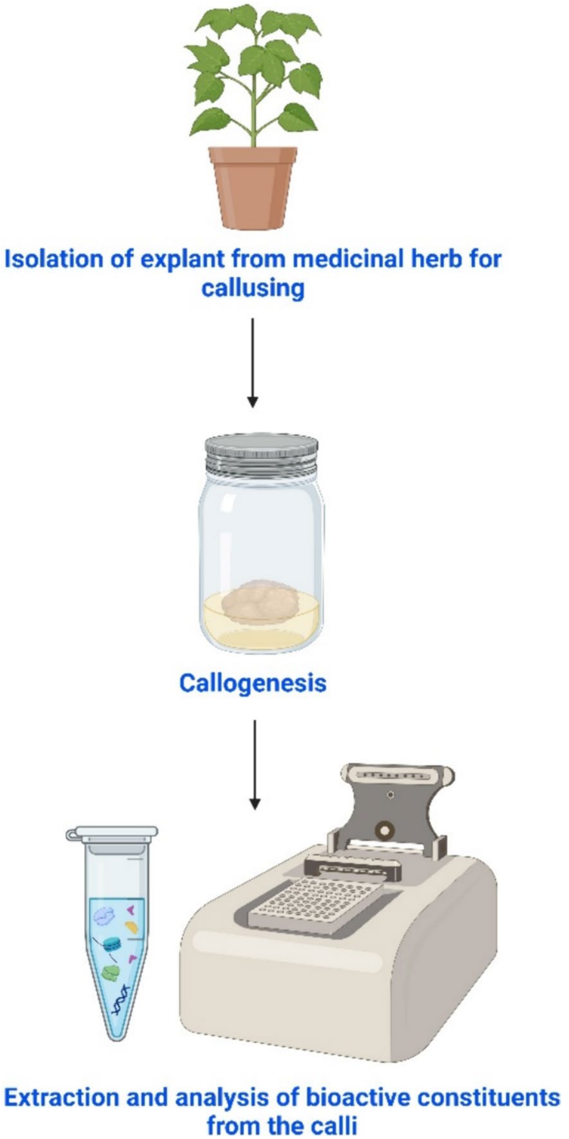
Physical attributes

The number of days taken to callus induction was counted after the inoculation of the explant on MS media. The developed calli of each medicinal plant were weighed five weeks after the explant's inoculation using an electronic scale (PR Series, Ohaus, USA). The diameter of the calli was also recorded five weeks after inoculation of explants using a vernier caliper (IP67, BEAPO Hardware Industrial Company, China). The percentage of callus induction was computed after 5th week of explant's inoculation using the below formula:

$$\textit{Callus induction \%} = \frac{\textit{Number of explants callusing}}{\textit{Total number of explants in the culture}} \times 100$$

Biochemical attributes

The calli of all experimental medicinal plants were subjected to biochemical compound extraction (Scheme 1). To find total phenolic content (TPC) in the calli of the experimental medicinal plants the absorbance was noted at 765 nm wavelength using



Scheme 1 Schematic diagram of in vitro extraction of enzymatic and non-enzymatic antioxidants from medicinal herbs

Folin-Ciocalteu reagent (Ainsworth and Gillespie 2007). A standard curve was plotted at the end of the test to obtain the gallic acid concentration, and TPC was expressed in mg kg^{-1}. The calculation of the flavonoid level was achieved by using method reported by Haider et al. (2024). In short, a 1 ml sample of callus culture from the experimental medicinal plants was thoroughly homogenized with 4 ml of deionized water and 300 µl of sodium nitrite. Then, the samples were stored for five minutes. Subsequently, a solution containing 2 ml of 1M NaOH was stirred with 300 µl of $AlCl_3$. The absorbance was carried out at 510 nm wavelength. It was expressed as mg kg^{-1}.

The antioxidative enzyme activities were analyzed by extracting 1 g of callus tissue in 2 ml phosphate buffer of pH 7.2 with the help of a chilled mortar and pestle. Then the mixture was centrifuged for five minutes at 4°C with the help of a Rotofix 46 centrifuge (Hettich, Kirchlengern, Germany) set at 10,000×*g*. After a collection of supernatants, the activities of antioxidative enzymes were determined. The enzymatic activities of ascorbate peroxidase (APX) (EC 1.11.1.11), catalase (CAT) (EC 1.11.1.6), peroxidase (POD) (EC 1.11.1.7), and superoxide dismutase (SOD) (EC 1.15.1.1) were quantified using the methodology described in a recent study by Haider et al. (2023). The samples underwent analysis at multiple wavelengths: 290 nm for APX, 240 nm for CAT, 470 nm for POD, and 560 nm for SOD. The enzyme activities were measured and expressed in µmol kg^{-1} FW.

The 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH-RSA) was determined using the methodology outlined by Ali et al. (2023), and the results were represented as a percentage of inhibition. In order to evaluate antioxidant capacity of selected medicinal plants callus, the methodology adopted by Osman et al. (2022) was used. The molybdate reagent was prepared using 1 ml of 4 mM $(NH_4)_2MoO_4$, 28 mM Na_3PO_4, and 0.6 M H_2SO_4. Then, the volume was increased to 50 ml with distilled water. The calli of all medicinal herbs were extracted after homogenization process. Subsequently, 100 ml of the supernatant was pipetted into a test tube. The test tube contained a total volume of 4 ml, including 3 ml distilled water and 1 ml molybdate reagent. The test tube was incubated at 95 °C for 90 min. Following that, the test tube was allowed to cool until it reached the surrounding temperature, which took about half an hour. The resultant reaction mixture's absorbance was then measured at 695 nm wavelength. The average values were measured and the findings were reported in micromoles equivalents of Trolox per gram of fresh callus weight of the sample. For the ABTS assay, a previously described protocol by Shahzad et al. (2022) was adopted and results were expressed as mg of Trolox equivalent in one gram of extract (mg TE g^{-1} extract).

Statistical analysis

Data was processed in Microsoft Excel 2016. The analysis of variance (ANOVA) of processed data was performed by using a Microsoft Windows application, Statistix 9® (Analytical Software, Tallahassee, USA). The means pair-wise analysis was achieved through Least Significant Difference Test. The correlation among the measured attributes was examined using "corrplot" function of R program 4.0.2 through the general linear model procedure (R Core Team 2022). A significance level of 5% was chosen for all the above statistics.

Table 1 Analysis of variance for experimental medicinal herbs for days to callus initiation (DCI), callus induction percentage (CIP), callus weight (CW), and callus diameter (CD)

Source of variance	DCI	CIP	CW	CD
	Percentage of total variance			
Medicinal herbs (MH)	85.26*	84.70**	93.23**	38.30**
Error	14.74	15.30	6.77	61.70

*Significant at $P \leq 0.05$

**Significant at $P \leq 0.01$

Results and discussion

Physical measurements of calli

There were significant differences ($P \leq 0.05$) between experimental medicinal plants for the studied physical attributes including days to callus initiation, callus induction percentage, fresh callus weight, and callus diameter (Table 1). Datura, goji berry, and carnation took the shortest period (13 days) to start callusing, followed by harmal (16.3 days), whereas, the longest period (20 days) was taken by bitter cucumber (Fig. 1a). Likewise, callus induction percentage was also observed highest (98.7%) in datura, followed by

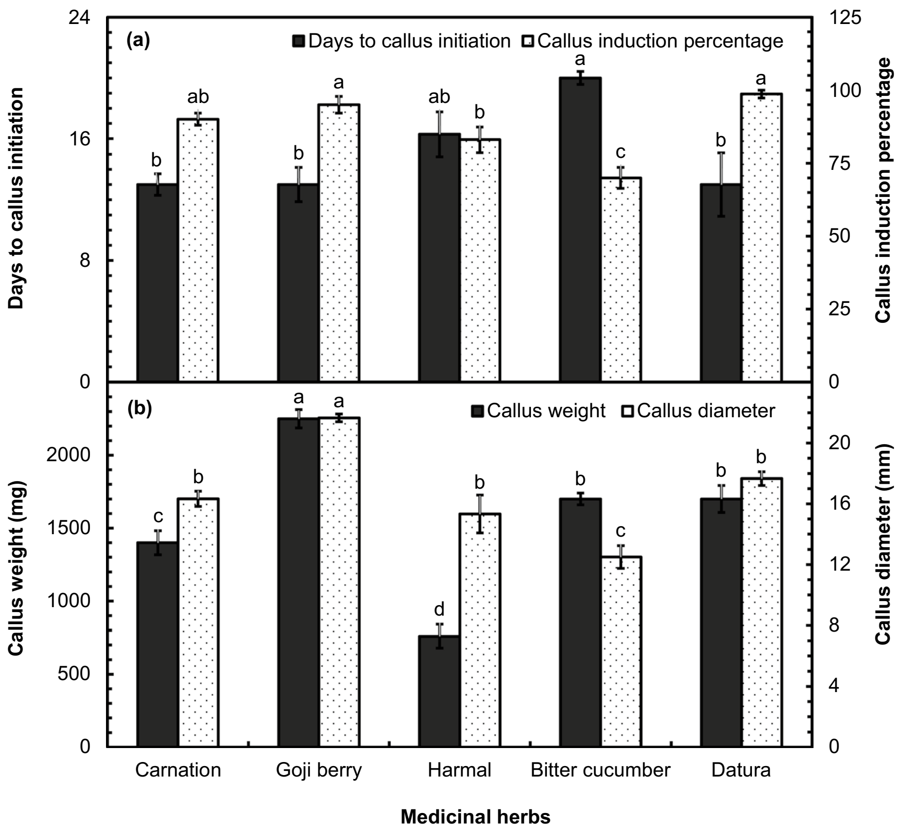


Fig. 1 Comparison of five medicinal herbs for days to callus initiation and callus induction percentage (**a**) and callus weight and callus diameter (**b**). Vertical bars show the means' standard error (±) (n = 4). The letters given above the vertical bars represent the statistical variations between the treatment means performed through the least significant difference test at $P \leq 0.05$ following analysis of variance. NS, non-significant

goji berry (95%), carnation (90%), and harmal (83%), while, the lowest callus induction was observed in bitter cucumber (70%) (Fig. 1a). In the present study, the highest rate of callus induction was observed in those leaf explants of datura supplemented with 1 mg L^{-1} BAP and 2 mg L^{-1} 2,4-D together in Murashige and Skoog (MS) media (Fig. 2). This could be due to presence of endogenous hormones in the explants which determine their callus induction ability (Das et al. 2018; Javed et al. 2023). The synergistic impact of BAP with 2,4-D have been reported for callus induction by several authors (Arif et al. 2014; Prakash et al. 2014; Das et al. 2018; Wang et al. 2023). The variations in callus responses among different medicinal plants with varied doses of plant growth regulators (PGRs) is possibly due to variations in endogenous PGRs levels in the explants. In case of combined use of cytokinin + auxin such as BAP + 2,4-D in goji berry and datura, and kinetin + NAA in carnation, the least callus initiation time (13 days) was recorded in the explants and these combinations were noticed to be ideal for callus induction in previous reports of Mathur and Shekhawat (2013) and Ardestani et al. (2015) showing that type and concentration of PGRs required for callus induction varies between plant species. The callus of goji berry was also found heaviest (2250 mg) followed by that of datura (1700 mg) and bitter cucumber (1700 mg), produced significantly similar mass of callus, and then carnation (1400 mg) (Fig. 1b). The lightest callus was produced by harmal (760 mg) (Fig. 1b). The maximum diameter (15.67 mm) was observed in callus of goji berry, followed by that of datura (15.3 mm), carnation (15 mm), and harmal (14 mm) (Fig. 1b). The minimum diameter was observed in the callus of bitter cucumber (12.5 mm) (Fig. 1b). Our study confirms the previous findings reported by Xu et al. (2008) and Prakash et al. (2014) that the ratio of cytokinin to auxin is crucial for callogenesis and subsequent callus growth.

Analysis of non-enzymatic and total antioxidants in fresh calli

Total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid content (AAC), and total antioxidant capacity (TAC) were highly significant ($P \leq 0.01$) as influenced by five different experimental medicinal plant species (Table 2). TPC was found highest in callus of goji berry (415 mg g^{-1}), followed

Fig. 2 Calli induction in selected medicinal herbs including carnation (**A**), goji berry (**B**), harmal (**C**), bitter cucumber (**D**), and datura (**E**) by standardizing their Murashige and Skoog media composition protocols

Table 2 Analysis of variance for experimental medicinal herbs for total phenolic content (TPC), total flavonoid content (TFC), enzyme activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), ascorbic acid content (AAC), total antioxidant capacity (TAC), 2,2-diphenyl-1-picrylhydrazyl-radical scavenging assay (DPPH-RSA), and 2,2-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) in their fresh calli

Source of variance	TPC	TFC	CAT	SOD	POD	APX	AAC	TAC	DPPH-RSA	ABTS
	Percentage of total variance									
Medicinal herbs (MH)	95.68**	85.92**	97.25**	87.36**	93.78**	94.50**	96.41**	94.67**	95.77**	95.77**
Error	4.32	14.08	2.74	12.62	6.21	5.49	3.58	5.32	4.22	4.22

**Significant at $P \leq 0.01$

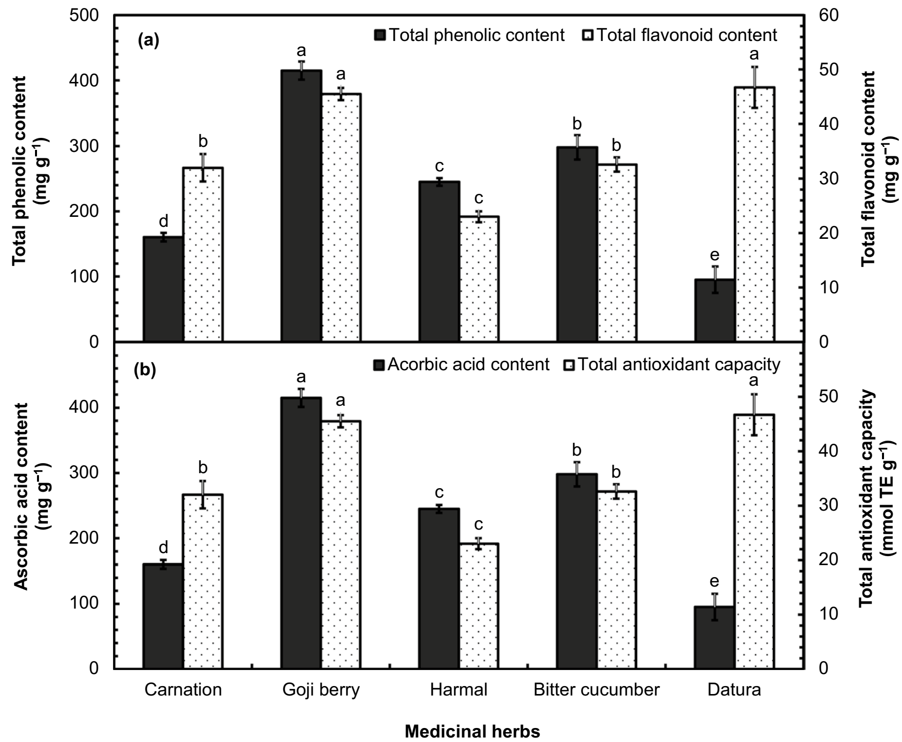


Fig. 3 Comparison of five medicinal herbs for total phenolic and total flavonoid content (**a**) and ascorbic acid content and total antioxidant capacity (**b**) in their fresh calli. Vertical bars show the means' standard error (±) (n = 4). The letters given above the vertical bars represent the statistical variations between the treatment means performed through the least significant difference test at $P \leq 0.05$ following analysis of variance

by that of bitter cucumber (298 mg g^{-1}), harmal (245 mg g^{-1}), and carnation (160.3 mg g^{-1}) (Fig. 3a). The lowest TPC (95.2 mg g^{-1}) was traced in callus of datura (Fig. 3a). The results are in line with Sharifzadeh et al. (2023) and Fabros et al. (2023), who found highest TPC in the callus raised on MS media containing cytokinin and auxin together. In the present study, callus weight and diameter had a strong positive correlation (> *0.8*) with TPC (Fig. 4). The results are in good agreement with Nazir et al. (2020) and Anwar et al. 2024, who observed a positive relationship between callus biomass and TPC. However, our finding contradicts with Loredo-Carrillo et al. (2013) who found an inverse relationship of phenolic content of *Azadirachta indica* with callus weight. Alternatively, TFC was found highest (46.7 mg g^{-1}) in callus of datura, followed by that of goji berry (45.5 mg g^{-1}), bitter cucumber (32.6 mg g^{-1}), and carnation (32 mg g^{-1}) (Fig. 3a). The lowest TFC was noted in harmal (23 mg g^{-1}) (Fig. 3a). It is evident from many previous reports that BAP is the most appropriate source of cytokinin to regulate cell division in medicinal plants and 2,4-D auxin for the initiation and proliferation of callus (Farhadi et al. 2017; Farvardin et al. 2017; Basit et al. 2023). In this study, the

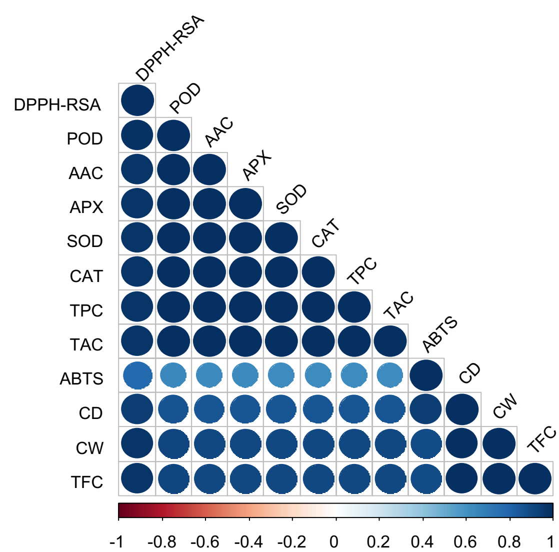


Fig. 4 Pearson correlation ($P \leq 0.05$) among the analyzed physicochemical attributes of five selected medicinal herbs. CW, callus weight; CD, callus diameter; TPC, total phenolic content; TFC, total flavonoid content; AAC, ascorbic acid content; CAT, catalase; SOD, superoxide dismutase; POD, peroxidase; APX, ascorbate peroxidase; DPPH-RSA, 2,2-diphenyl-1-picrylhydrazyl-radical scavenging activity; ABTS, 2,2-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid; TAC, total antioxidant capacity

highly productive callus for TFC belonged to datura which was raised on MS media comprising 1 mg L^{-1} BAP + 2 mg L^{-1} 2,4-D. The results are remarkably similar with Palacio et al. (2012) who noted that differentiation of plant tissues is a prerequisite for production of flavonoids. Similarly, Karakas and Turker (2016) found that highly-proliferated and compact plant callus contained higher levels of secondary metabolites. AAC (415 mg g^{-1}) and TAC (46.7 mmol TE g^{-1}) were found higher in the calli of goji berry and datura, respectively (Fig. 3b). On the other hand, lowest levels of AAC (95.2 mg g^{-1}) and TAC (23 mmol TE g^{-1}) were noted in datura and harmal (Fig. 3b). Krishnan et al. (2015) have reported similar results that endogenous ascorbic acid content differs among plant species and that cytokinin and auxin have a synergistic effect on callus's antioxidant levels.

Analysis of radical scavenging assays and enzymatic antioxidants in fresh calli

The radical scavenging assays including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) as well as enzyme activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) were significantly affected by medicinal plant species (Table 2). The values of DPPH-RSA (387.2%) and ABTS (267.8 mg TE g^{-1}) were found highest in calli of goji berry, followed by bitter cucumber, harmal, and carnation (Fig. 5a). The lowest values of DPPH-RSA (68.4%) and ABTS (47.3 mg TE g^{-1}) were recorded in calli of datura (Fig. 5a). In line with our results, Krishnan et al. (2015) observed higher DPPH-RSA and ABTS in callus culture of *Gynura procumbens* than *G. bicolor*. This may be attributed to the variations in the scavenging effect among plant family and species, whereby plants of same family *Pseudopiptadenia contorta* and *Platypodium elegans* exhibited different scavenging effects (Mensor et al. 2001). In an earlier study, leaf callus cultures of *Ocimum sanctum* showed a range of variation for antioxidant activity in the form of DPPH-RSA and ABTS (Song et al. 2012). CAT (393.4 µmol s^{-1} kg^{-1}) and SOD (112.7 µmol s^{-1} kg^{-1}) enzyme activities were found highest in calli of goji berry, while datura was found to have lowest activities of both CAT (151.4 µmol s^{-1} kg^{-1}) and SOD (47.1 µmol s^{-1} kg^{-1}) enzymes in its calli (Fig. 5b). Although, calli of goji berry and harmal were statistically similar for CAT enzyme activity (Fig. 5b). Similarly, calli of datura and bitter cucumber were also observed to have significantly similar enzyme activities of SOD (Fig. 5b). Furthermore, goji berry exhibited highest activities of POD (456.7 µmol s^{-1} kg^{-1}) and APX (367.6 µmol s^{-1} kg^{-1}) enzymes (Fig. 5c). The lowest enzyme activities of POD (222.6 µmol s^{-1} kg^{-1}) and APX (151 µmol s^{-1} kg^{-1}) were recorded in datura and bitter cucumber, correspondingly (Fig. 5c). Catalase is a key enzymatic H_2O_2 scavenger that catalyzes the breakdown of H_2O_2 into H_2O and O_2 (Libik et al. 2005). Our study supports the findings of Fikret et al. (2013) and Saeed et al. (2024), who noted a substantial cultivar variation in the antioxidative enzyme activities of eggplant's callus tissues. However, our study refutes the findings of Cui et al. (1999), who reported that the production of callus in *Lycium barbarum* has always been preceded by a decline in CAT activity. SOD is main scavenger of O^{-2} that catalyzes superoxide radical into H_2O_2 which is further scavenged by CAT and other peroxidases (Lin and Kao 2000). Fikret et al. (2013) found much higher SOD activity in callus of salt-tolerant eggplant cultivar than salt-sensitive. Furthermore, Karakas et al. (2016) observed a strong positive correlation between CAT, SOD, POD and APX activities and accumulation of total phenolics, total flavonoids which is in good agreement with our findings as evident from Fig. 4. Overall, the findings of this research provide a strong empirical confirmation of goji berry callus as a potential source of enzymatic and non-enzymatic antioxidants.

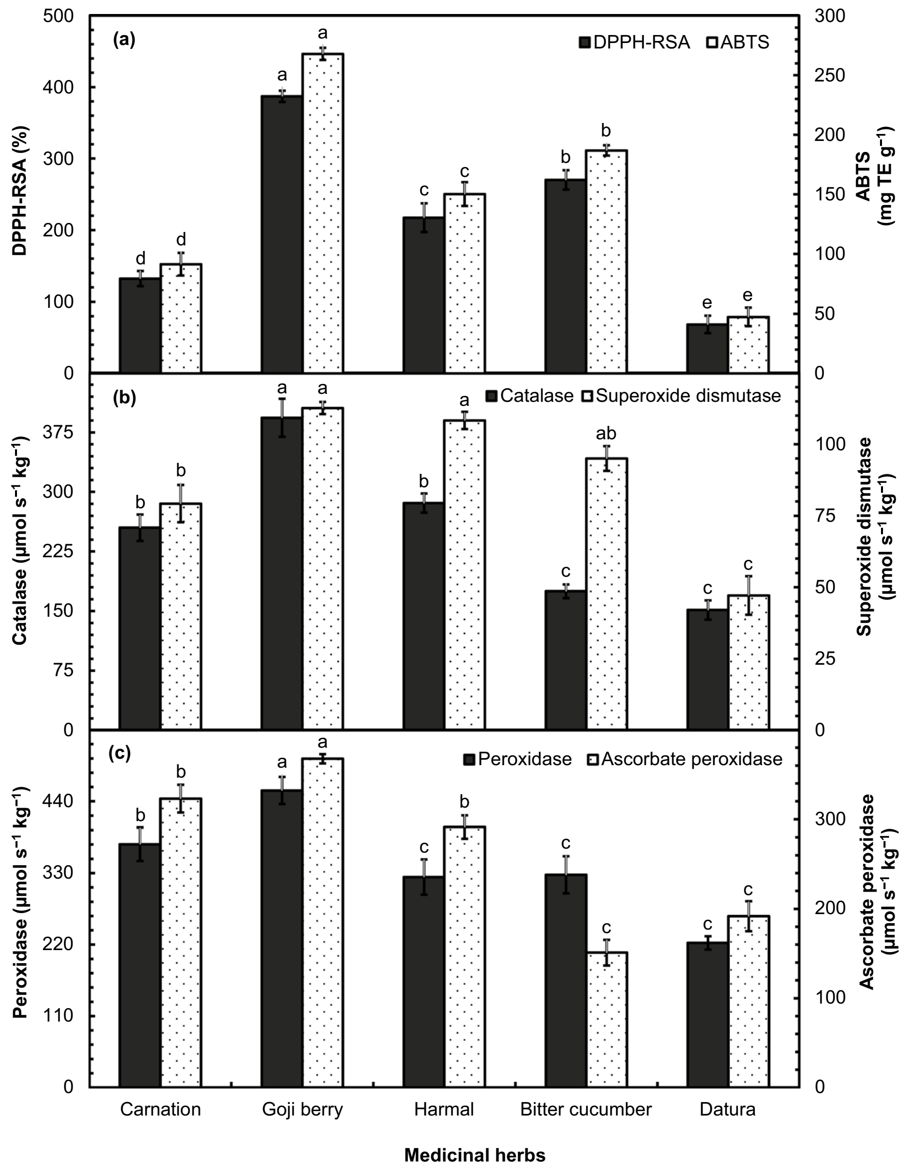


Fig. 5 Comparison of five medicinal herbs for 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH-RSA) and 2,2-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) (**a**), catalase and superoxide dismutase (**b**), and peroxidase and ascorbate peroxidase (c) enzyme activities in their fresh calli. Vertical bars show the means' standard error (±) (n = 4). The letters given above the vertical bars represent the statistical variations between the treatment means performed through the least significant difference test at $P \leq 0.05$ following analysis of variance

Conclusion

Our study has established a foundation for modern research to apply new biotechnologies on metabolic pathways in order to reveal ideal conditions for calli induction in potential medicinal plants and maximize the bioactive compounds production. Furthermore, our findings have proved that the callus of goji berry is an abundant source of natural antioxidants for their sustainable supply to the pharmaceutical industry.

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Author contributions M.W.H., M.N. and M.B.T. designed the sampling strategy. M.W.H. and M.N. designed the experiments. M.B.T. performed the experiment. M.N. provided materials and supervision. M.W.H. wrote the manuscript. U.F., T.H., T.D., A.M., M.S.R., A.M.A.M., H.R., O.S., T.A., A.A., and R.I, reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability All the data related to this work can be sourced from the corresponding authors.

Declarations

Conflict interest The authors declare no competing interests.

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