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Research Article

Enhancing phytochemical content and bioactive aspects in somatic embryogenesis developed from callus of *Phoenix dactylifera* L.

Ammar M.A. ALI[1,2*], Ahmed A. QAHTAN[3], Jameel M. AL-KHAYRI[4*], Methaq N. ALGABR[2], Othman M. AL-DOSSARY[4], Bader ALSUBAIE[4]

[1]*Hajjah University, Faculty of Education, Department of Biology, Hajjah, Yemen; ammarsood21@gmail.com (*corresponding author)*
[2]*Modern Specialized University, Faculty of Medical Sciences, Department of Pharmacy, Sana'a 72738, Yemen; amar@msu.edu.ye; dr.methaq@msu.edu.ye*
[3]*King Saud University, College of Science, Department of Botany and Microbiology, Riyadh 11451, Saudi Arabia; ahmadaqq@gmail.com*
[4]*King Faisal University, College of Agriculture and Food Sciences, Department of Agricultural Biotechnology, Al-Ahsa 31982, Saudi Arabia; jkhayri@kfu.edu.sa (*corresponding author); othmand@kfu.edu.sa; subaiebys@kfu.edu.sa*

Abstract

This research aims to investigate the chemical profile of methanolic (MeOH) extracts of callus and somatic embryogenesis (SE) induced from date palm 'Barhi' cv. (*Phoenix dactylifera* L.) by total polyphenols estimation and gas chromatography-mass spectrometry (GC-MS) analysis. In addition, some biological aspects as antiradical and anti-enzymes inhibitory effects were evaluated by *in vitro* examinations. Results demonstrated that, total phenol content (TPC) and total tannin content (TTC) were significantly increased by 14.95% and by 16.83%, respectively, in callus than that estimated in SE. Whereas total flavonoid content (TFC) in SE recorded a very high increase rate of 564.4% above that found in callus. GC-MS chromatogram of SE extract had more phytochemicals about 35 molecules were identified, including five specific bioactive compounds with considered ratios ranging between 2.265% and 12.76%. Overall, the determined biological aspects were significantly improved ($p < 0.05$) in SE, particularly anti-Diphenyl-2-picryl-hydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Total Antioxidant Capacity (TAC) by 48%, 92.31%, and 54.35% sequentially as antiradical/antioxidant potentials in terms of IC_{50} values. Furthermore, anti-α-amylase and anti-α-glucosidase effects were enhanced by 2,750% and 224.16%, respectively, in SE compared to callus results. In summary, MeOH extract of SE was richer in phytochemicals with greater bioactive properties than callus; this evaluated superiority could be attributed to the developmental and morphological differentiation occurring for SE tissues, which may be a prerequisite for greater content production and accumulation of many types of bioactive secondary metabolites. Further prospective pharmacological studies are required to recommend SE of 'Barhi' cv. as a natural nutritional and therapeutic source.

Keywords: chemical profile; date palm; *in vitro* regeneration; pharmaceutical properties; plant tissue culture

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Introduction

Dates are a popular fruit of *Phoenix dactylifera* L. plant, considered a functional food due to its highly nutritional and medicinal values (Hussain *et al.*, 2020; Krueger, 2021; Fernández-López *et al.*, 2022; Al-Mssallem *et al.*, 2024). Many rural people, particularly those in developing countries, consume dates as main meal, specifically to nurture children suffering from malnutrition and food shortages. Dates represent a rich food source of high-energy components, minerals, and dietary fibers (Johnson *et al.*, 2015). Additionally, dates are used to treat many ailments, such as gastric, neurodegenerative, and cardiovascular diseases (Calderon-Montano *et al.*, 2011; Vayalil, 2012).

There are hundreds of cultivars of date palm growing in the Mediterranean area, and some are very famous due to their high nutritional value (Habib and Ibrahim, 2011; Zhang *et al.*, 2017). 'Barhi' cv. is one of those famous fruit crops in the Mediterranean area, and it was reported to have superior nutritional and physiochemical features, such as high fructose and microelements amounts, better antioxidant aspect, and very low anti-nutrient contents among 11 date palm cultivars grown in the United Arab Emirates (Rambabu *et al.*, 2020). Also, Perveen and Bokahri (2020) suggested 'Barhi' is one of the most common of three dates grown in Saudi Arabia for treating mineral deficiency. With these valuable aspects, the demand for dates is increasing annually, which has led to an increase in date prices. Also, the rise in dates prices has been accompanied by many problems facing the expansion of date palm cultivation by conventional approaches.

As a result, date palm is commonly propagated by offshoots, which are only produced by the mother plant in a limited number of daughter offshoots (Mazri and Meziani, 2015). Also, date palm is constantly infected with many diseases and pests, which cause significant yield loss of this cultivar. These factors constitute valid reasons to search for solutions to these problems. Plant tissue culture as a modern technique for producing unlimited and healthy plants *in vitro* is the first choice and the appropriate tool in this scale.

Callus represents the primary stage to initiate many forms of *in vitro* cultures as indirect organogenesis, somatic embryogenesis, regardless of changes environmental conditions (Thingbaijam and Huidrom, 2014). Also, callus is always used as a sustainable and steady source to produce many secondary metabolites and bioactive components (Ali *et al.*, 2022). Somatic embryogenesis (SE) is a biotechnological system employed frequently to establish biomass of embryos directly or indirectly for *in vitro* cultures of many plants, and this technique represents an attractive tool to study the changes occurring during the development of somatic embryos as morphological, biochemical, cytological, and genetic alterations (Bellaj and Hadrami, 2004; Morel *et al.*, 2014; Zein Eldin and Ibrahim, 2015).

Previously, different date palm varieties, including 'Barhi' cv., were successively *in vitro* propagated by inducing somatic embryos from different explants, mainly from shoot tips (Al-Khayri, 2011; Jazinizadeh *et al.*, 2015; Awadh *et al.*, 2019; Al-Asadi *et al.*, 2024; Al-Mayahi *et al.*, 2024), mature inflorescence (Kriaa *et al.*, 2012) and immature inflorescence (Taha *et al.*, 2021). There is still a notable lack of research on the biochemical and biological characteristics of date palm *in vitro* cultures, particularly regarding the changes that occur during different developmental stages.

Some studies were conducted on the determination of some biochemical contents and sometimes bio-aspects as antioxidants of certain kinds of cultures established *in vitro* as embryogenic callus/non-embryogenic callus (Aslam *et al.*, 2011), somatic and zygotic embryos (Zein Eldin and Ibrahim, 2015), cell suspension culture (Al-Khayri and Naik, 2022), embryogenic and degenerative embryogenic calli (Zein El Din *et al.*, 2021), and somatic embryos (Zein El Din *et al.*, 2022) was reported in some date palm cultivars. Therefore, and based on what was concluded above, some biochemical examinations as GC-MS analysis as well as many pharmacological investigations especially anti-enzyme inhibitory effects during initiation and developmental stages of date palm in *in vitro* conditions especially of 'Barhi' cv., have still not addressed. For this purpose, our study was designed to investigate the phytochemical profile by total polyphenol molecules estimation and GC-MS analysis, as well as the antiradical and anti-enzyme inhibitory potentials were evaluated in callus culture and their indirect SE of date palm 'Barhi' cv.

Materials and Methods

The *in vitro* cultures experiments were implemented during 2021-2022 in the Plant Tissue Culture Laboratory-General Corporation for Agricultural Services in Sana'a, Republic of Yemen. Phytochemicals characterization and biological evaluation were carried out in the Department of Botany & Microbiology, College of Science, King Saud University.

Plant materials

Five date palm offshoots of healthy 'Barhi' cv. free of diseases and pests' characters were selected and then collected from date palm farms in Al-Jawf Province, Republic of Yemen, in 2020.

Explant preparation

To prepare shoot tip explants for *in vitro* planting, consecutive steps were implemented. First, the tough and fibrous outer leaves of offshoots were cut off with a hacksaw until the softer cores were visible, then a sharp knife was used to remove carefully the inner soft leaves around the bud located in the offshoot pith. After that, the shoot tip explants were isolated, and the sterilizing protocol was applied inside a laminar flow cabinet by immersing the obtained shoot tips in a 0.1% mercuric chloride solution for 30 min, then the explant was rinsed three times in sterile distillate water. After that, antioxidant treatment was applied by soaking the explant in an antioxidant solution that contained 150 and 100 mg L^{-1} of ascorbic and citric acids, respectively, until immersing the sterilized explants in callus medium.

Callus induction and proliferation

To induce callus, the sterilized explants were prepared for *in vitro* planting by cutting each one longitudinally into four equal pieces and inserting each segment individually in a test tube containing solid MS (Murashige and Skoog, 1962) medium fortified with formulations of plant growth regulators (PGRs) as described in Table 1, which conclude the treatments applied of different concentrations and combinations of PGRs in callus cultures (induction and proliferation). The cultures were incubated for 6 weeks. Then, the best medium induced callus was determined (by measuring callus fresh weight), and about 0.3 g of induced callus was proliferated three times (4-week interval) on the MS medium augmented with 1 mg L^{-1} 2,4-D + 5 mg L^{-1} 2iP as that reported by Al-Khayri and Naik (2022). All cultures were incubated in the dark at 25 ± 2 °C.

Table 1. Basal MS mediums augmented with PGRs for callus induction and proliferation

Culture stage	Treatment	PGRs in basal MS medium mg L^{-1}			
		2.4-D	BA	NAA	2iP
Callus initiation	1	10	-	-	3
	2	50	-	-	3
	3	-	-	30	3
	5	0	0	0	0
Callus proliferation	1	1	-	-	5

Somatic embryogenesis formation

To establish indirect SE from shoot tips derived callus tissue, the methodology described by Zein El Din *et al.* (2021) was followed with minor modifications implemented. Briefly, about 0.5 g jar^{-1} of white or creamy induced calli was selected as a source to initiate the embryogenic callus on MS medium augmented with 0.1 mg L^{-1} NAA for 12 weeks (4-week interval) in the dark, then the obtained friable embryogenic callus was sprouted on MS medium augmented with 0.1 mg L^{-1} NAA + 0.05 mg L^{-1} BA for 12 weeks in the dark. After that the germinated embryogenic callus was re-cultured on similar medium used for embryos germination but the incubation was in 8/16 h light/dark to induced the primal green shoots.

Methanolic (MeOH) extract preparation

Approximately 50 g of FW of callus and SE was freeze-dried, then the obtained yield of dried materials was weighed (4.6 g dry weight of callus and 5.2 g dry weight of SE) and powdered by a blender. After that, 4 g of each powder was dissolved individually in 50 mL of 99% methanol. The extraction process was performed at 30 ± 2 °C, pH 7±0.5 and 150 rpm for 12 h using an orbital shaker, and subsequently filtered using Whatman No. 1 filter paper. The filtrated extract was dried at room temperature and stored at 4 °C until use. Callus and SE fresh and dry weight and the yield of extracts are demonstrated in Table 2.

Table 2. Fresh and dry weight and extractive yields of callus and SE of date palm 'Barhi' cv.

Plant material	Fresh weight (g)	Dry weight (g)	MeOH extract yield (mg)	Extractive yield (%)
Callus	50	4.6	45	0.98
SE	50	5.2	59	1.13

Phytochemical analysis

Total Phenolic Content (TPC)

To estimate the TPC in the studied extracts, the methodology of Velioglu *et al.* (1998) was followed with slight modifications. About 0.75 mL of 10% (v/v) Folin-Ciocalteu reagent was mixed with 100 µL of either MeOH extract or gallic acid. The mixture was combined with 0.75 mL of a 60 g L^{-1} sodium bicarbonate solution and allowed to rest at 25 ± 2 °C for 30 min in the dark, pH of mixture was adjusted to be around 7 ± 0.2 A UV-visible spectrophotometer was then used to measure the mixes' absorbance at 725 nm. The results were represented as mg gallic acid equivalent g^{-1} plant dry weight (mg GAE g^{-1} DW).

Total Flavonoid Content (TFC)

The flavonoid quantification process was carried out using the procedure outlined by Barajas-Ramírez *et al.* (2023) with minor adjustments. Briefly, about 300 µL of 1 M potassium acetate and 1.5 mL of extract (1 mg mL^{-1}) were mixed with 10% (w/v) aluminium chloride reagent. After 30 min of dark incubation at 25 ± 2 °C, pH 7 ± 0.2 the samples' absorbance was determined spectrophotometrically at 415 nm. The results were given as mg of equivalent quercetin g^{-1} of plant dry weight (mg QE g^{-1} DW).

Total Tannin Contents (TTC)

The TTC in examined samples was evaluated by the method described by Chandran and Indira (2016) with slight adjustments. A mixture of 1.5 mL of distilled water, 0.2 mL of a 35% (w/v) sodium carbonate solution, 100 µL of Folin-Ciocalteu reagent, and 1 mg mL^{-1} of MeOH extract or tannic acid were freshly prepared. Then, the mixture's absorbance after an incubation period in the dark at 25 ± 2 °C for 1 h and pH 7 ± 0.2, the mixture was measured at 700 nm using a UV-visible spectrophotometer. The results were represented as mg of tannic acid equivalent g^{-1} of plant dry weight (mg TAA g^{-1} DW*).*

GC-MS analysis

GC-MS analysis was performed for the MeOH extracts of the tested samples by gas chromatography coupled with the mass spectrometry analytical method as reported by Ali *et al.* (2022). Briefly, GC-MS analysis was performed using Rtx-5MS capillary column (5% diphenyl-95% dimethylsilicone, 30 m × 0.25 mm ID × 0.25 µm), and a temperature program was 50 °C for 1 min ramped to 300 °C for 3 min. Identification of compounds was based on a comparison of mass spectra with the GC-MS system data bank (NIST 08 library), a comparison with published data, and retention indices. The relative amount of each compound was expressed as percent peak area relative to the total peak area of the GC chromatogram.

Antioxidant Activities

DPPH assay

The DPPH assay was used to evaluate the DPPH scavenging activity in the tested extracts according to the methodology outlined by Sogi *et al.* (2013). The standard curve was created using ascorbic acid (AC) dissolved in water at levels ranging from 1 to 32 μg mL^{-1}. AC equivalents (mg AAE g^{-1} DW) per gram of dry sample weight were used to express the results.

ABTS radical scavenging assay

The ABTS+ radical cation de-colorization assay was used to measure the ABTS scavenging activity according to Tang *et al.* (2020) methodology. Ascorbic acid equivalents (AAE) per gram (mg AAE g^{-1} DW) of samples were used to express the antioxidant ability. The AC calibration curve was created and shown at various doses within standard limits of 0.78 - 25 μg mL^{-1}.

TAC determination

Assessment of TAC for different tested samples was implemented according to the method of Prieto *et al.* (1999). TAC was expressed as mg of AC equivalents g^{-1} of extract (mg AAE g^{-1} DW).

FRAP assay

The technique outlined by Kenari *et al.* (2014) was used to calculate FRAP of different examined samples. The FRAP results were expressed as mg of AC equivalents g^{-1} of extract (mg AAE g^{-1} DW).

Enzyme inhibition activity

To estimate the enzyme suppression capacity of the examined extracts inverse α-amylase and α-glucosidase enzymes activity, the approach described by Zengin (2016) was followed. α-glucosidase and α-amylase were expressed as acarbose equivalents (ACE).

Statistical analysis

All experiments, except GC-MS analysis, were run in triplicate, and the results were expressed as mean ± standard deviation (SD) values. Results were statistically analysed using SPSS version 20 to investigate if there are any significant difference (alternative hypothesis) or no significant differences (null hypothesis) between the outcomes of studied samples (callus and SE) at $p < 0.05$. One-way ANOVA-Duncan's post hoc test was selected (as common test used to compare between more than two means/groups) to investigate the significant differences between mean values of phytochemicals, antioxidants, and enzymatic inhibitory properties of MeOH extracts of callus and SE of date palm 'Barhi' cv. at $p < 0.05$.

Results

Effect of different formulations of PGRs on callus induction and proliferation

Results in Table 3 show the effect of different formulations of PGRs on callus induction and proliferation, a significant effect was exerted on callus-induced shoot tip explants when the auxin 2.4-D/NAA was used in low or high levels combined with a constant level (3 mg L^{-1}) of cytokinin 2iP, compared to the control treatment when no callus was observed (Figure 1A). The lower concentration used of either 2,4-D (10 mg L^{-1}) or NAA (30 mg L^{-1}) induced more values of callus FW 0.279 ± 0.17 mg and 0.198 ± 0.27 mg, respectively (Figures 1B and 1C), compared to less amounts of callus FW 0.223 ± 0.32 mg and 0.185 ± 0.86 mg, which were induced by a higher level of 50 mg L^{-1} of either 2.4-D or NAA sequentially (Figures 1D and 1E). Different colours and similar texture of callus were observed with the low and high levels of auxin treatments; a compact texture with creamy-coloured callus appeared in the medium containing lower auxin

levels (Figures 1B and 1C), whereas compact brown-coloured callus was formed when the higher levels of auxins were employed (Figures 1D and 1E). The optimum MS media formulation was that supplementing with 10 mg L^{-1} 2.4-D + 3 mg L^{-1} 2iP which induced significantly ($p < 0.05$) the highest fresh weight of callus, 0.279 ± 0.17 mg, compared to other treatments applied (Table 3 and Figure 1B).

Table 3. Effect of PGRs formulations on callus induced from shoot tip explants of date palm ('Barhi' cv.)

Culture stage	Treatment	PGRs in basal MS medium mg L^{-1}			Parameter (Callus)		
		2.4-D	NAA	2iP	Fresh weight (mg)	Color	Texture
Callus initiation	1	10	-	3	0.279 ± 0.17 a	Creamy	Compact
	2	50	-	3	0.223 ± 0.32 b	Brown	Compact
	3	-	30	3	0.198 ± 0.27 c	Creamy	Compact
	4	-	50	3	0.185 ± 0.86 c	Brown	Compact
	5	0	0	0	0.117 ± 0.10 d	-	-

Different letters within the column in table indicate a significant difference at $p < 0.05$.

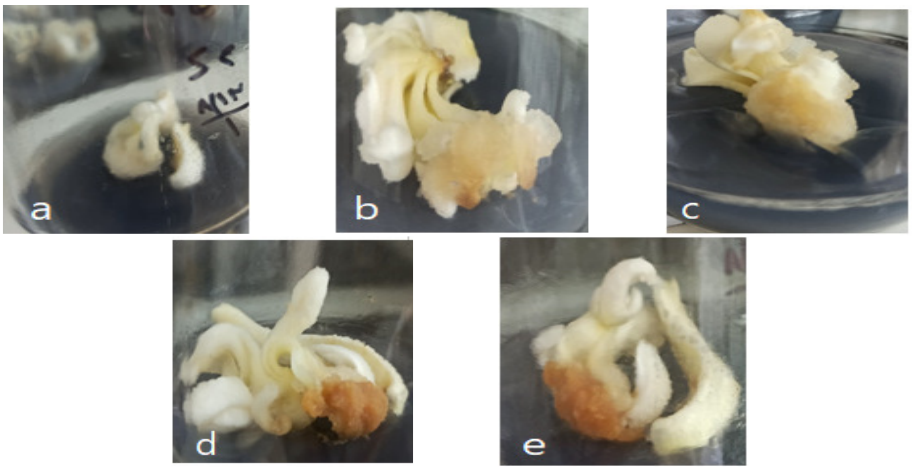

Figure 1. Callus induction on MS medium supplemented with (A) 0.00 control (B) 10.00 mg L^{-1} 2.4-D + 3 mg L^{-1} 2iP, (C) 30 mg L^{-1} NAA + 3 mg L^{-1} 2iP, (D) 50 mg L^{-1} 2.4-D + 3 mg L^{-1} 2iP, (E) 50 mg L^{-1} NAA + 3 mg L^{-1} 2iP

Callus was vigorously proliferated when compact creamy callus was cultured on medium (MS) fortified with 1.0 mg L^{-1} 2.4-D + 5.0 mg L^{-1} 2iP for three months in the dark condition at room temperature. With these *in vitro* treatments and conditions, undifferentiated cells in callus tissues were frequently divided till enough amounts of vigorous calli, about 100 g, were obtained with a friable texture and creamy colour were observed (Figure 2 A).

Somatic embryogenesis formation

Morphological development of somatic embryogenesis during six months of *in vitro* culture is depicted in Figures 2A-2F. Somatic embryos were primarily formed as small white granules that initiated from aggregation of cells in embryogenic callus (Figure 2B) as clusters (Figure 2C) during culture on MS medium + 0.1 mg L^{-1} NAA for 12 weeks in the dark (as described by Zein El Din *et al.*, 2021). A piece of this distinct tissue was then germinated on MS medium augmented with 0.1 mg L^{-1} NAA + 0.05 mg L^{-1} BA for 12 weeks in the dark, which developed into small, white buds with formation of 6.2 ± 0.96 bud/tube (Figures 2D and 2E).

These sprouts were then re-cultured on the same medium but incubated in 8/16 h light/dark to form green shoots for 1 month (Figure 2F).

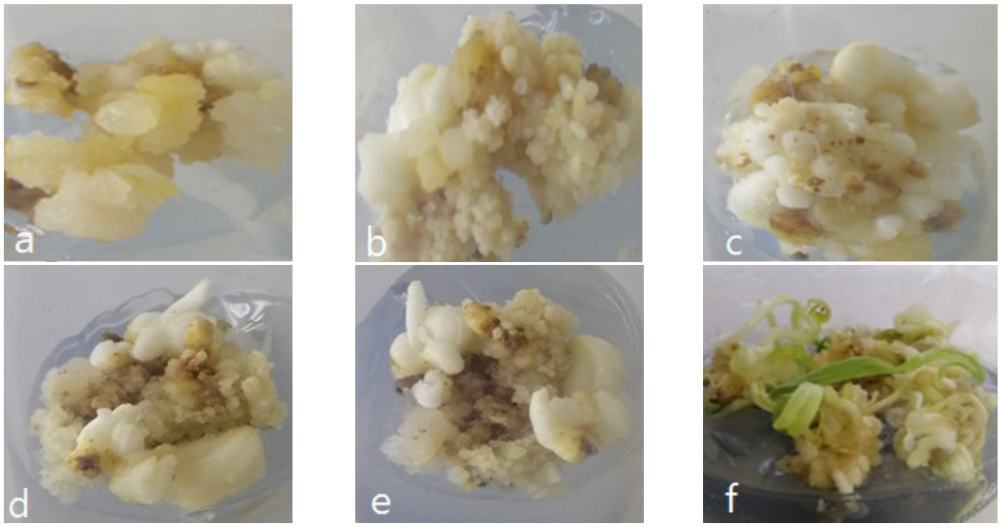

Figure 2. Morphological development progress of SE of date palm 'Barhi' cv. during 7 months of culture (A) friable callus, (B) embryogenic callus, (C) initiated somatic embryos as clusters of aggregated cells, (D, E) germinated somatic embryos as small sprouts, (F) development of green shoots

Phytochemical analysis

TPC, TFC, and TTC estimation

Quantitation of total polyphenols (TPC, TFC, and TTC) was determined for MeOH extracts of tested samples, callus and SE and their results are presented in Table 4 which showed higher contents of TPC (37.12 ± 0.51 mg GAE g^{-1} DW) and TTC (16.03 ± 0.87 mg TAA g^{-1} DW) in callus extract which recorded significant ($p < 0.05$) increases of 14.95% in TPC and 16.83% in TTC than that amounted in the extract of SE. The TFC in SE extract recorded a very high increasing percentage of 564.40% compared to that measured of TFC in callus extract.

Table 4. Total phenol, flavonoid and tannin contents in MeOH extracts of callus and SE of date palm 'Barhi' cv.

Tested sample	TPC (mg GAE g^{-1} DW)	TTC (mg TAA g^{-1} DW)	TFC (mg QE g^{-1} DW)
Callus	37.12 ± 0.51a	16.03 ± 0.87 a	3.92 ± 0.38 a
SE	32.29 ± 0.63 b	13.72 ± 0.59 b	0.59 ± 0.32 b

Different letters within the same column in table indicate a significant difference at $p < 0.05$

GC-MS analysis

GC-MS profile of MeOH extract of callus was determined and compared to SE results. Table 5 shows different characters of components identified by GC-MS analysis in MeOH extracts of callus and SE, twenty-three components were characterized in MeOH extract of callus, and this extract was dominated by glycoside with the highest content 45.43% was the amount of ethyl α-d-glucopyranoside molecule, which formed the major component in callus; fatty acids were the second class identified, which comprised 23.26% of the total callus extract, followed by 10.45% of monosaccharide desulphosinigrin molecule and 5.72% of piperazine 1-nitroso, whereas fatty acids esters formed about 3.64% of the total percent of callus extract; other class/phytochemicals were determined in low or trace amounts. Concerning the GC-MS of SE extract, more phytochemicals, approximately 35 components, were characterized, and SE extract was also dominated by the ethyl α-d-glucopyranoside molecule but with a lower percent, 27.56%, compared to that detected in callus; the

fatty acids esters were determined with a higher percent, 27.81%, whereas fatty acids were detected with a lower percent, 17.70%, compared to that reported in callus. About 6 phytomolecules were specific components to MeOH extract of SE and not detected in callus; these components were detected with considered ratios and were previously reported as bioactive components such as DL-proline, 5-oxo-, methyl ester (12.76%), 2-Pyrrolidinone (6.416%), hexadecanedioic acid, methyl ester (3.939%), 4H-Pyran-4-one, 2,3-dihydro-3.5-dihydroxy-6-methyl (3.44%), and linoelaidic acid (2.265%). Figure 3, demonstrates the correlation among GC-MS components of callus and SE based on the level of component, represented by the gradual colours from dark red to pale blue.

Table 5. GC-MS profile of MeOH extracts of callus and SE of date palm 'Barhi' cv.

No.	RT (min)	Name of compound	Area %		Molecular weight (g mol^{-1})	Structural formula
			Callus	SE		
1	8.185	Dl-Allo-cystathionine	-	0.135	222	$C_7H_{14}N_2O_4S$
2	8.862	2-pyrrolidinone	-	6.41	85	C_4H_7NO
3	9.108	Piperazine, 1-nitroso-	5.72	-	115	$C_4H_9N_3O$
4	9.527	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	-	3.44	144	$C_6H_8O_4$
5	9.8	Imidazole,2-amino-5-[(2-carboxy) vinyl]	0.246	-	153	$C_6H_7N_3O_2$
6	9.813	Mannosamine	-	0.71	179	$C_6H_{13}NO_5$
7	10.085	Debrisoquine	2.11	-	175	$C_{10}H_{13}N_3$
8	10.206	Acetamide, N-methyl-N-[4-(3hydroxypyrrolidinyl)-2-butynyl]-	-	0.85	210	C11H18N2O2
9	10.328	Pterin-6-carboxylic acid	1.813	-	207	$C_7H_5N_5O_3$
10	10.379	1-Deoxy-d-arabitol	-	2.88	136	C5H12O4
11	10.897	2-Methyl-9-β-d-ribofuranosylhypoxanthine	-	2.74	282	$C_{11}H_{14}N_4O_5$
12	11.428	DL-Proline, 5-oxo-, methyl ester	-	12.76	143	$C_6H_9NO_3$
13	11.429	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester)	2.56	-	197	$C_9H_{11}NO_4$
14	12.011	Deoxyspergualin	1.657	-	387	$C_{17}H_{37}N_7O_3$
15	12.022	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester)	-	1.175	197	$C_9H_{11}NO_4$
16	12.171	1H-Purin-2-amine, 6-methoxy-N-methyl-	-	0.422	179	C7H9N5O
17	12.369	Deoxyspergualin	-	2.04	387	C17H37N7O3
18	13.122	Ethyl α-d-glucopyranoside	45.43	27.56	208	$C_8H_{16}O_6$
19	13.702	2-Bromotetradecanoic acid	0.931	-	306	$C_{14}H_{27}BrO_2$
20	13.878	Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-	-	2.072	252	C14H24N2O2
21	14	2-(Ethylenedioxy)ethylamine,N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-	-	1.045	252	C14H24N2O2
22	14.126	Benzenemethanol, 3,4,5-trimethoxy-	-	2.084	198	C10H14O4
23	14.286	Acetamide, N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-	1.973	0.813	266	$C_{14}H_{22}N_2O_3$
24	14.477	4-Methoxycarbonylmethylundec-3-enedioic acid, dimethyl ester	-	0.811	314	$C_{16}H_{26}O_6$
25	14.602	Hexadecanoic acid, methyl ester	-	3.406	270	$C_{17}H_{34}O_2$
26	14.66	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	-	1.019	292	C18H28O3
27	14.71	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	-	0.569	416	$C_{27}H_{44}O_3$
28	14.78	N-Hexadecanoic acid	8.74	3.29	256	$C_{16}H_{32}O_2$
29	14.906	Desulphosinigrin	10.449	-	279	$C_{10}H_{17}NO_6S$
30	15.045	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester	-	3.847	374	$C_{25}H_{42}O_2$

31	15.297	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	0.405	0.66	322	$C_{21}H_{38}O_2$
32	15.483	Linoleic acid	2.798	3.727	280	$C_{18}H_{32}O_2$
33	15.485	7,10-Octadecadienoic acid, methyl ester	0.446	-	294	$C_{19}H_{34}O_2$
34	15.517	Trans-13-Octadecenoic acid	6.747	3.939	282	$C_{18}H_{34}O_2$
35	15.519	Cis-Vaccenic acid	0.593	3.788	282	$C_{18}H_{34}O_2$
36	15.65	Heptadecanoic acid, 9-methyl-, methyl ester	0.226	0.331	298	$C_{19}H_{38}O_2$
37	15.705	Linoelaidic acid	-	2.265	280	$C_{18}H_{32}O_2$
38	15.858	Octadecanedioic acid	1.64	0.49	314	$C_{18}H_{34}O_4$
39	16.213	Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl	-	0.447	254	$C_{15}H_{26}O_3$
40	16.825	Ethyl iso-allocholate	-	0.244	436	$C_{26}H_{44}O_5$
41	16.994	Hexadecanedioic acid	-	0.29	286	$C_{16}H_{30}O_4$
42	18.919	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-	0.857	330	$C_{19}H_{38}O_4$
43	21.722	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester	-	0.755	354	$C_{21}H_{38}O_4$
44	21.805	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-	2.088	356	$C_{21}H_{40}O_4$
45	21.903	1-Hexadecanol, 2-methyl-	2.39	-	256	$C_{17}H_{36}O$
46	24.213	L-Gala-l-ido-octose	0.19	-	240	$C_8H_{16}O_8$
47	26.608	10-octadecenal	0.475	-	266	$C_{18}H_{34}O$
48	27.336	I-Propyl 9-tetradecenoate	1.522	-	268	$C_{17}H_{32}O_2$
49	32.761	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	1.297	-	416	$C_{27}H_{44}O_3$
Total area			**100**	**99.96**		

Antioxidant potential effect

Anti-DPPH and anti-ABTS activities

Antiradical action against DPPH and ABTS radicals was estimated for MeOH extracts of callus and SE. Results in Table 6 show a gradually increasing of anti-radical effect against DPPH and ABTS radicals, which was associated with elevating the examined concentrations of both extracts from 0.031 mg mL^{-1} to 2 mg mL^{-1}, with the highest antiradical effects was 89.72 ± 0.60 mg AA g^{-1} and 88.39 ± 0.81 mg AA g^{-1} being evaluated for 2 mg mL^{-1} of SE extract against DPPH and ABTS radicals, respectively. MeOH extract of callus showed less inhibition effects at all levels employed, particularly against DPPH free radicals, which was lower ($p < 0.05$) than that estimated of the similar concentrations of SE extract. IC_{50} values of anti-DPPH and anti-ABTS activities were also estimated for both extracts of callus and SE. The highest IC_{50} value was that of anti-ABTS in both SE and callus extracts, with a higher IC_{50} value of 0.304 mg g^{-1} for the SE extract than that resulted by the callus extract IC_{50} = 0.365 mg g^{-1}, and a non-significant difference ($p > 0.05$) was calculated between both IC_{50} values evaluated.

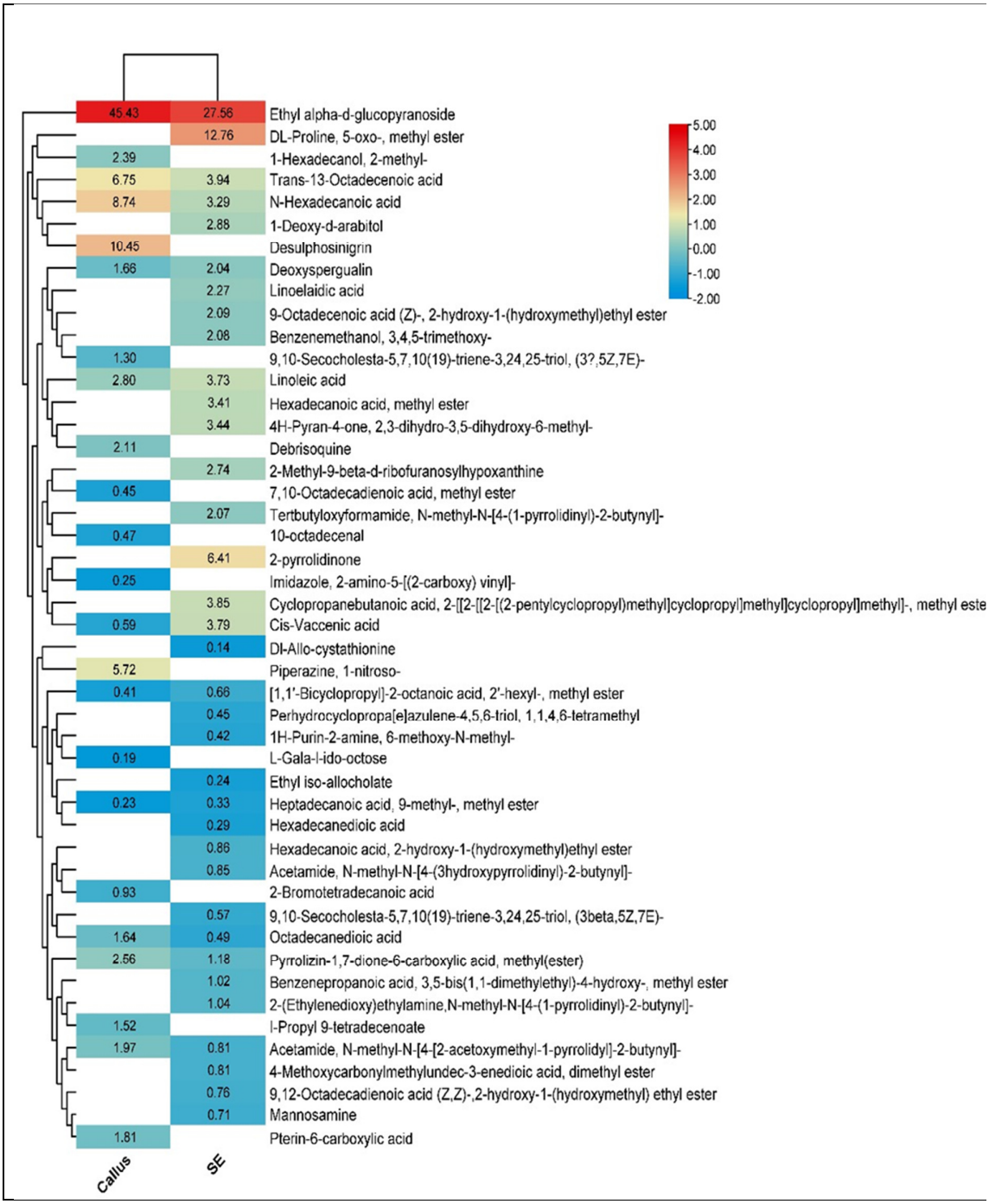

Figure 3. Cluster heatmap analysis based on relative phytochemicals levels of *P. dactylifera* 'Barhi' cv. (calls and somatic embryogenesis; SE) as determined by GC-MS. The colours in the matrix boxes indicate the strength and direction of correlation

Table 6. Anti-DPPH and Anti-ABTS free radicals scavenging activities of callus and SE of date palm 'Barhi' cv.

MeOH extract (Conc. mg mL^{-1})	Anti-ABTS (mg AA g^{-1})		Anti- DPPH (mg AA g^{-1})	
	Callus	SE	Callus	SE
0.031	8.18 ± 0.43 a	9.71 ± 0.66 a	2.16 ± 0.75 b	8.43 ± 0.14 a
0.063	21.57 ± 1.27 a	22.28 ± 0.13 a	8.23 ± 0.67 c	14.59 ± 0.59 b
0.125	24.76 ± 1.28 a	26.89 ± 0.21 a	11.21 ± 0.69 c	19.94 ± 0.63 b
0.25	31.39 ± 1.11 b	36.37 ± 0.25 a	19.22 ± 0.15 c	22.92 ± 0.38 c
0.5	52.49 ± 0.88 b	58.18 ± 1.60 a	24.05 ± 1.05 d	38.23 ± 0.84 c
1	74.18 ± 0.85 b	79.98 ± 1.30 a	50.06 ± 1.13 d	64.03 ± 0.64 c
2	84.96 ± 1.01 b	88.39 ± 0.81 a	72.46 ± 0.88 c	89.72 ± 0.60 a
IC_{50} value (mg g^{-1})	**0.365 a**	**0.304 b**	**0.893 c**	**0.464 b**

Different letters within the same row in table indicate a significant difference at $p < 0.05$.

FRAP and TAC effects

The results obtained from MeOH extracts of callus and SE by FRAP and TAC assays were consistent and confirmed the results of ABTS and DPPH experiments. The results depicted in Figure 4 show that the higher antioxidant potential was measured for SE extract in both assays (FRAP and TAC), which was increased by 92.31% and 54.35% of FRAP and TAC potentials, respectively, than that evaluated in callus extract.

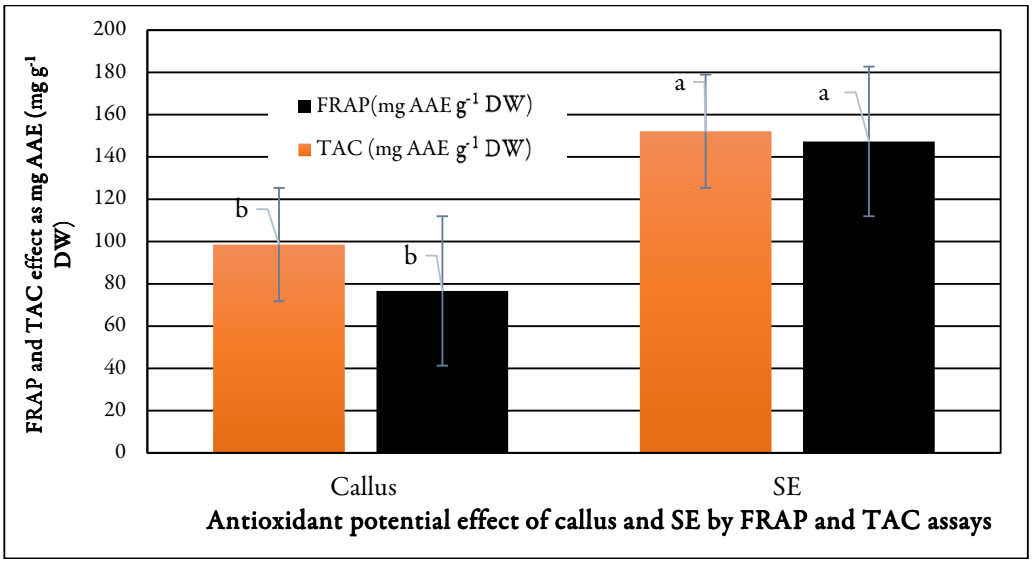

Figure 4. FRAP and TAC of MeOH extracts of callus and SE of date palm 'Barhi' cv. Different letters on the same-coloured column indicate a significant difference at $p < 0.05$

Correlation value

A strong correlation was detected between antioxidant potentials of plant extracts in all experiments applied and TFC with $R^2 = 0.99$, and non-correlations were estimated with TPC and TTC $R^2 = -1$.

Anti-enzyme inhibitory effects

The enzyme inhibitory properties of MeOH extracts of shoot tip-derived callus and SE were evaluated against α-amylase and α-glucosidase enzymes; results are summarized in Table 7. Overall, the obtained results showed that, the enzymes suppression effects were significantly enhanced ($p < 0.05$) when the callus was

developed to SE tissues. MeOH of SE recorded higher anti-α-amylase and anti-α-glucosidase activities by an increasing percent of 2750% and 224.16%, respectively, than that measured in MeOH of callus.

Table 7. Anti-enzyme inhibitory effects of MeOH extracts of callus and SE of date palm 'Barhi' cv.

Tested sample	α-amylase inhibition (mmol ACAE g^{-1}extract)	α-glucosidase inhibition (mmol ACAE g^{-1} extract)
SE	0.57 ± 0.015 a	4.83 ± 0.13 a
Callus	0.02 ± 0.024 b	1.49 ± 0.09 b

Discussion

Plant biotechnology includes various techniques widely employed with unlimited benefits for humans, animals, and their environmental surroundings. Plant tissue culture is one of the major plant biotechnological tools that contributes to solving many problems associated with classical agriculture and enables researchers to study plants at different growth stages with the possibility of implementing varied manipulations and then measuring their results under controlled conditions. In the present work, the changes in the phytochemical profile and some bioactive aspects were evaluated in callus culture established from date palm shoot tips and SE developed from callus tissues.

Callus induction and proliferation

A significant increase in FW of callus was recorded when the treatments contained lower concentrations 10 mg L^{-1} of 2.4-D or 30 mg L^{-1} of NAA compared to higher levels of auxin (30 mg L^{-1} of 2.4-D or 50 mg L^{-1} of NAA), with a superior callus FW value of 0.279 ± 0.17 g, was significantly ($p<0.05$) achieved by the medium containing10 mg/L of 2.4-D with the presence of 3.0 mg L^{-1} 2iP, which formed the optimum formulation of PGRs for callus initiation. Also, our findings showed that no callus was formed in the control treatment. These findings indicated that the presence of PGRs (auxin and cytokinin) is essential during callus induction and proliferation. Due to the synergistic effect exerted by the two types of PGR, auxin could play an important role in cell dedifferentiation and biomass accumulation, whereas cytokinin has a considerable effect in cell division during callogenesis (Sané *et al.*, 2006; Baharan and Mohammadi, 2018; Al-Khayri and Naik, 2022). This desired effect is only obtained when an appropriate equilibrium is applied between the auxin and cytokinin combination (Murthy *et al.*, 2014; Baharan and Mohammadi, 2018). In previous reports, a similar formulation of PGRs (2.4-D at 10 mg L^{-1} + 3 mg L^{-1} of 2ip) was reported repeatedly as the optimum treatment for inducing better/more generative callogenesis from shoot tip explants of different cultivars of date palm, such as 'Sewi' cv. (Zein Eldin and Ibrahim, 2015), 'Barhi' cv. (Zein El Din *et al.*, 2021), and 'Sewi' cv. (Zein El Din *et al.*, 2022). These findings are consistent with our results. Similarly, the results of Al-Khayri and Naik (2022) study confirmed that, that higher concentrations of auxin and cytokinin combination reduced biomass accumulation in cell suspension cultures of the 'Shishi' cv.

Somatic embryogenesis formation

Somatic embryos were successively formed indirectly during three sequential stages: initiation, germination, and maturation by MS medium fortified with optimized formulations of PGRs as described by Zein El Din *et al.* (2021). MS medium + 0.1 mg L^{-1} NAA was used to induce undifferentiated cells from friable callus, which aggregate as clusters with formation of white granules (primary formation of differentiated tissues) in dark conditions during 8 weeks, and then the obtained differentiated cells were transformed to MS medium augmented with 0.1 mg L^{-1} NAA + 0.05 mg L^{-1} BA for another 8 weeks, also in the dark for germination, which formed small white buds, and these were regenerated on the same medium and were developed to small green seedlings when they were exposed to 8/16 h.light/dark. In the present work and

during initiation somatic embryos, there were two types of calluses, white friable callus and brown callus were formed, as that resulted in Zein El Din *et al.* (2021) study. The former one (white friable callus) was only selected for SE formation while the latter (brown callus) was avoided due to that, this callus was degenerative and characterized with high content of some inhibitory agents as phenols, H2O2, MDA, and DHA, which may lead to weak or no embryo formation. Also, in the present work, all stages of embryogenesis were successfully developed in the dark. Abohatem *et al.* (2023) showed that, the time required for development indirect SE into plantlets in date palm was significantly decreased by 50% in full darkness incubation.

Phytochemical content

TPC, TFC and TTC in callus and SE

Results of total polyphenols estimation demonstrated greater amounts of TPC and TTC were significantly ($p < 0.05$) accumulated in callus MeOH extract with an increasing ratio 14.95% was measured in TPC and 16.83% in TTC than that determined in the MeOH extract of SE. In contrast, SE had significantly more content (3.92 ± 0.38 mg QE g^{-1} DW) of TFC, which was higher by 564.40% compared to a trace amount (0.59 ± 0.32 mg QE g^{-1} DW) of TFC was evaluated in callus extract. In previous studies, different results were reported as increasing/decreasing the total bioactive compounds content during different stages of *in vitro* cultures of many date palm cultivars. In the study conducted by Zein Eldin and Ibrahim (2015), a gradually increasing ($p < 0.05$) was measured in the levels of phenols and flavonoids during development *in vitro* cultures of date palm ('Sewi' cv.) from the embryogenic callus stage to the zygotic embryo stage. Also, biochemical levels of some primary metabolites like protein and amino acids were increased during the development of somatic embryogenesis in six cultivars of date palm, including the 'Barhi' cv. whereas sugar content was decreased, the obtained findings were imputed to the variation in the level of endogenous PGRs and maturity/development of somatic embryo (Aslam *et al.*, 2011). The results recorded by Zein El Din *et al.* (2021) mainly ascribed the qualitative and quantitative variation measured in phenolic compounds of *P. dactylifera* 'Barhi' cv. during embryogenic callus formation to the type of callus tissue established. Also, other factors, such as culture conditions, type, and formulations of PGRs, could play a critical role in the biosynthesis and accumulation of phytochemicals during *in vitro* cultures. It was reported that, the type of PGRs is one of the major influencers not only on cell growth and differentiation but also secondary products synthesis and accumulation is also influenced, particularly phenols and flavonoids (Murthy *et al.*, 2014; Al-Khayri and Naik, 2022). Many studies found that the maximum accumulation of polyphenol content was detected in *in vitro* culture growing on medium augmented with auxin 2,4-D alone or in combination with cytokinin, as that reported in date palm cell suspension cultures of 'Shishi' cv. (Al-Khayri and Naik, 2022) and other plants, such as Cnidium officinale callus culture (Adil *et al.*, 2018) and callus culture of Garcinia brasiliensis (Teixeira *et al.*, 2019). These findings supported our results when the highest TPC and TTC were estimated in callus initiated and proliferated on 2,4-D-containing medium. Whereas NAA was preferred to produce flavonoids (Mamdouh and Smetanska, 2022). This was also observed in our results when the higher content of TFC was amounted in SE grown on medium fortified with NAA. Similar findings were previously reported by Mamdouh and Smetanska (2022) when the highest TFC were evaluated in callus of *Lycium schweinfurthii* induced on multiplication media containing NAA.

GC-MS analysis

The MeOH extracts of the *in vitro* established callus and SE were characterized, and various types and different contents of constituents were identified by GC-MS investigation. GC-MS results of callus extract revealed the presence of 23 constituents, whereas 35 phyto-constituents were identified in SE extract. Some types of major molecules identified were similar in both extracts of callus and SE, but with different content, such as ethyl α-d-glucopyranoside (glycoside), which formed about 45.431% of the total percent of callus extract, and this content was decreased to be 27.565% in SE extract. Ethyl α-d-glucopyranoside was detected in

GC-MS of callus in some plants, such as ginger, with a higher amount of 92.96% (Ali *et al.*, 2022). The detected component was reported as a good preservative (Rajalakshmi and Mohan, 2016; Chavan and Santa-Catarina, 2023). Also, other bioactive components formed the main molecules identified in both examined extracts but with lower levels than the first main molecule. These components were some fatty acids, such as n-hexadecanoic acid (8.740% in callus and 3.295% in SE), trans-13-octadecenoic acid (6.747% in callus and 3.939% in SE), and linoleic acid (2.798% in callus and 3.727% in SE). These determined fatty acids were detected in considered amounts in different varieties and many parts of ex-vitro date palm (Perveen and Bokahri, 2020; Hamzah *et al.*, 2024). And in callus culture of other plants, as callus of *Dipterygium glaucum*, when GC-MS examination revealed the presence of n-Hexadecanoic acid (palmitic acid) as one of the major components detected with 6.47% (Choudhary *et al.*, 2019). Seeds and callus essential oils of *Argania spinosa* have high contents of linoleic acids 25-36.8% respectively (Koufan *et al.*, 2020). Varied pharmacological activities were previously reported for n-hexadecanoic acid as anti-cancerous (Pascual *et al.*, 2017), anti-inflammatory (Aparna *et al.*, 2012), antioxidants, 5-α reductase inhibitors, and insecticides properties (Sermakkani and Thangapandian, 2012). Also, linoleic acid had a wide range of bioactive aspects, including anti-cancer, anti-coronary, anti-inflammatory, and 5-α reductase inhibitor (Ponnamma and Manjunath, 2012; Chirumamilla *et al.*, 2022). A lot of other main detected active components were specific/identified only in callus or in SE extract. In callus desulphosinigrin (monosaccharides) formed about 10.449% of the total callus content; this component was not detected in SE. Desulphosinigrin was one of six major compounds in cell suspension cultures of *Hybanthus enneaspermus* with 13.03% (Krupashree *et al.*, 2018). Many pharmacological properties were ascribed to desulphosinigrin as potential antidiabetic drug against α-glucosidase (Sari *et al.*, 2023) and anticancerous properties (Saravanan *et al.*, 2014). Concerning GC-MS chromatogram of MeOH extract of SE, more phyto-constituents (35 components) were detected, and the extract was also dominated by other distinguished/specific components such as DL-Proline, 5-oxo-, methyl ester (12.76661%), 2-Pyrrolidinone (6.416328%), hexadecanedioic acid, methyl ester (3.93912%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (3.44%), and linoelaidic acid (2.265446%). Some of these components were characterized in the GC-MS chromatogram of MeOH extract of callus of different plants, such as callus of *Merremia dissecta*, which had about 13.35% of DL-Proline, 5-oxo-, methyl ester (Joshi *et al.*, 2018), and 2-Pyrrolidinone was detected in callus of *Corbichonia decumbens* with 10.04% (Gomathi *et al.*, 2019), and also callus of *Rheum emodi* contained a considered concentration 8.82% of hexadecanedioic acid, methyl ester (Singh and Chaturvedi, 2019). While 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl was detected in ethanolic extract of non-embryogenic callus of *Eurycoma longifolia* with 7.068% (Iriawati *et al.*, 2014). Different bioactive aspects against some human cancer cell lines and antioxidants were reported for 2-Pyrrolidinone (Thangam *et al.*, 2013). And antibacterial and antifungal effects were reported for hexadecanoic acid, methyl ester (Chandrasekaran *et al.*, 2011). Whereas linoleic acid has anticancer effects against human breast cancer cell lines MCF-7 (Dutta *et al.*, 2023). And 4H-Pyran-4-one, 2.3-dihydro-3.5-dihydroxy-6-methyl was reported as a strong antioxidant (Yu *et al.*, 2013) and anti-inflammatory agent (Amala and Jeyaraj, 2014).

Based on the obtained findings of GC-MS analysis in this study, which proved the richness of MeOH extracts of callus and SE of 'Barhi' cv. with many bioactive constituents, in addition to that reported in previous studies of many health/profitable benefits for the detected components as α-d-glucopyranoside (natural preservative), n-hexadecanoic acid (antioxidant, anti-inflammatory, anti-cancerous, 5-α reductase inhibitors and insecticide), linoleic acid (anti-cancer, anti-coronary, anti-inflammatory, and 5-α reductase inhibitor), desulphosinigrin (antidiabetic), 2-Pyrrolidinone (antioxidant and anti-cancer), 4H-Pyran-4-one, 2.3-dihydro-3.5-dihydroxy-6-methyl (antioxidant and anti-inflammatory) and hexadecanoic acid, methyl ester (antibacterial and antifungal), our findings could indicate that MeOH extracts of both *in vitro* cultures, callus and SE are a multi-phytochemicals sources could provide isolation considered quantities of pure characterized components. These components may be contributed in production of many products belonging to different industrial fields as crude drugs for many chronic diseases and antiseptic materials (pharmaceutical industry), as natural preservatives (food industry) and insecticides for agricultural fields.

Bioactive properties

Antioxidant activities

Antioxidant potential of SE was greater than that evaluated in callus in different implemented assays when the anti-DPPH and anti-ABTS radicals scavenging potentials were enhanced in SE by 92.45% and 20.06% in terms of IC_{50} values estimated by DPPH and ABTS assays, respectively. Also, similar results for SE extract were estimated as increasing percent by 92.31% and 54.35% in FRAP and TAC potentials, respectively, then the effects of callus extract. These findings are in harmony with the results of phytochemical estimation, especially GC-MS results, which showed that SE extract contained significant and more types of specific bioactive components, which were detected as the main components, compared to fewer types of bioactive components that were characterized in callus extract. Despite that, callus extract had more content of TPC and TTC, but TFC was extremely increased by 564.40% in SE, and a strong positive correlation with $R^2 = 0.99$ was calculated between antioxidant potential and TFC. GC-MS chromatogram of SE extract was distinguished by the presence of a distinct flavonoid constituent, 4H-Pyran-4-one, 2.3-dihydro-3.5-dihydroxy-6-methyl, with considered ratio 3.44%; this molecule was reported as a strong antioxidant agent (Yu *et al.*, 2013). In the literature, only a few reports evaluated the antioxidant properties of some date palm *in vitro* cultures. Zein El Din *et al.* (2021) reported higher phenolic content with higher scavenging activity of embryogenic callus of date palm 'Barhi' cv. than that evaluated in degenerative embryogenic callus. Also, Al-Khayri and Naik (2022) results recorded the maximum accumulation of polyphenols with strong radical scavenging activity (90.65%) that were estimated in cell suspension cultures of date palm 'Shishi' cv. cultured on MS medium containing 5 mg L^{-1} 2.4-D and 2.5 mg L^{-1} 2ip. In other plants, various studies revealed superior antioxidant potential for SE in different stages than that of non/pre-embryonic callus, as that reported for SE of *Swertia corymbosa* (Mahendran and Narmatha Bai, 2017), somatic embryos of *Ajuga bracteosa* (Rukh *et al.*, 2019), and germinated SE of *Cotyledon orbiculata* (Zengin *et al.*, 2023). According to the results of these studies, which proved strong antioxidant potential effect with much polyphenolic content in SE of different plants, including some varieties of date palm, our findings are consistent with that previously demonstrated, when the greater antioxidant activities with richer content of bioactive constituents were estimated in SE of date palm 'Barhi' cv. than that in the earlier developmental phase (callus culture). This may indicate that, the *in vitro* SE stage may be an optimized phase of *in vitro* cultures of date palm was assessed with superior bioactive properties as potent antioxidant aspect with high accumulation of antioxidant molecules. These aspects could introduce SE of date palm as an immense and important antioxidant natural source for different applications in pharmacological, cosmetic and agronomic industries. Recently, a distinctive assortment of common antioxidant phenolic acid as coumaric, caffeic, gallic, vanillic, ellagic, and rutin, were detected in embryogenic callus of 'Barhi' cv. with higher amounts than that found in non-embryogenic callus (Zein El Din *et al.*, 2021). These components are frequently investigated in different plants as common bioactive contributors of a wide range of pharmacological properties, especially antioxidant activity. Therefore, a large-scale establishment of *in vitro* cultures of embryogenic callus and SE of 'Barhi' cv. may provide a continuous and regenerated crude source for preparation different forms of natural antioxidant products.

Anti-enzyme inhibitory potentials effect

Results of anti-enzymes inhibitory effects against α-amylase and α-glucosidase were significantly enhanced ($p < 0.05$) in SE, and the elevating ratio was 2,750% of the anti-α-amylase inhibitory effect and 224.16% of the anti-α-glucosidase inhibitory effect than that measured in callus. There is no report in the literature in terms of anti-enzyme inhibitory effects for any type of *in vitro* culture of date palm. Some studies reported anti-enzymes inhibition effects on α-glucosidase and α-amylase of date palm fruits (Famuwagun and Olasunkanmi Gbadamosi, 2021) and seeds (Khan *et al.*, 2016). Anti-enzymes inhibition investigation of *in vitro* culture was also lacking in the literature for the other plants; some reports, such as that recently conducted

by Zengin *et al.* (2023), showed potent acetylcholinesterase inhibitory activity for the mature SE extract of cotyledon *Cotyledon orbiculata* L. In the present work, the anti-enzymes inhibition effect against α-amylase and α-glucosidase was significantly enhanced ($p < 0.05$) in SE as that evaluated of antioxidant potential of than that in callus, and these greater bioactive aspects measured could be imputed to the richness of SE with many and different types of bioactive molecules, especially flavonoid molecules. These molecules were classified as the principal contributors of α-glucosidase and α-amylase inhibitory effects (Lam *et al.*, 2024).

Conclusions

As a result of the developmental and morphological differentiation that occurs in SE tissue, which may be a prerequisite for improving production and accumulation of large amounts and much variety of bioactive secondary phytochemicals in SE, the estimated bioactive aspects (antiradical and anti-enzymes suppression effects) were significantly enhanced in the MeOH extract of SE developed from callus. Based on that, SE of 'Barhi' cv. could be recommended as a natural antiradical and anti-diabetic product and may be used as a nutritional and pharmaceutical source after conducting further pharmacological and toxicological investigations.

Authors' Contributions

Conceptualization: AMAA; Investigation and Writing - original draft: AAQ; Methodology: JMA; Review and funding acquisition: MNA; Data curation: OMA; Editing: BA; Visualization.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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