Different indole-3-acetic acid and 6 benzyl amino purine concentrations affect biomass, phenolic profile, and bioactivity in Mentha rotundifolia L.

Article *in* Journal of Food Measurement and Characterization · June 2023

DOI: 10.1007/s11694-023-01974-w

CITATIONS
0

READS
214

10 authors, including:

Hadjer Kecis
biologie
4 PUBLICATIONS **2** CITATIONS
SEE PROFILE

Mohamed Bagues
Institut des Régions Arides
30 PUBLICATIONS **198** CITATIONS
SEE PROFILE

Fatiha Mekircha
University of Jijel
6 PUBLICATIONS **44** CITATIONS
SEE PROFILE

Lynda Gali
Biotechnology Research Center- CRBt Constantine ALGERIA
36 PUBLICATIONS **342** CITATIONS
SEE PROFILE

Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-023-01974-w

ORIGINAL RESEARCH

Different indole-3-acetic acid and 6 benzyl amino purine concentrations affect biomass, phenolic profile, and bioactivity in *Mentha rotundifolia* L.

Hadjer Kecis[1] · Mohamed Bagues[2] · Yahia Abdelouhab[1] · Fatiha Mekircha[3] · Lynda Gali[4] · Kenza Kadi[5] · Dalila Addad[6] · Kamel Nagaz[2] · Fatiha Brahmi[7] · Yacine Kouba[8]

Received: 1 March 2023 / Accepted: 11 May 2023

Abstract
Although the use of phytohormones for crop improvement has great potential, little is known about the molecular effects of phytohormones in crops. This work investigated the effect of the exogenous application of plant growth regulators (PGRs), indole-3-acetic acid (IAA), and cytokinin-like 6-benzyl amino purine (BAP) on plant biomass and the phytochemical and biological parameters of *Mentha rotundifolia* L. aerial parts and roots. The results showed that the application of IAA and BAP significantly influenced the accumulation of phenolic compounds in the plant organs. Treatment with PGRs also induced remarkable increases in the amounts of individual phenolic compounds, with the greatest increases observed for salvianolic and rosmarinic acids in the aerial parts treated with 20 or 10 mg/mL of BAP. Salvianolic acid also showed the most significant increase in the roots of plants treated at 10 mg/mL (from 18.232 to 41.317 µg/g of extract). Furthermore, enhanced antioxidant and inhibitory enzyme effects in the treated plants was observed. Our findings suggest that exogenous hormones could be used to improve the synthesis of phenolic compounds and, as a result, the bioactivity of medicinal or food plants.

Keywords *Mentha rotundifolia* L. · IAA · BAP · Exogeneous hormones · Phenolic compounds · LC-ESI-MS analysis · Antioxidant effect

Introduction

The genus *Mentha* contains aromatic plant species found in temperate climates such as the Mediterranean countries, Australia, and South Africa [1]. The species of this genus have received much interest because of their chemical composition (mainly essential oils and polyphenols) and their pharmacological effects [2, 3]. In particular, *M. rotundifolia* (synonym: *M. suaveolens* Ehrh Still; Family: Lamiaceae) is one of the six species of *Mentha* that grows spontaneously in Algeria, commonly known as "timarssad" and widely used as a spice or infusion, decoction, and hydrolat in traditional medicine for a variety of effects including tonic, carminative, digestive, stomachic, antispasmodic and sedative [4, 5].

✉ Hadjer Kecis
h.kecies@centre-univ-mila.dz

1 Laboratory of Natural Science and Materials (LSNM), Institute of Science and Technology, Abdelhafid Boussouf University Centre, Mila, Algeria

2 Laboratory of Drylands and Oases Cropping, Arid Regions Institute of Medenine, University of Gabes, Medenine, Tunisia

3 Laboratory of Biotechnology, Environment and Health, Faculty of Natural and Life Sciences, University Mohammed Seddik Ben Yahia, 18000 Jijel, Algeria

4 Biotechnology Research Center—CRBt, Constantine, Algeria

5 Laboratory of Biotechnology, Water, Environment, and Health, University of Abbes Laghrour, Khenchela, Algeria

6 Department of Ecology, SNV Faculty, Abbes Laghrour University, Khenchela, Algeria

7 Laboratory of Biomathematics, Biophysics, Biochemistry and Scientometry, Faculty of Natural Sciences and Life, University of Bejaia, 06000 Bejaia, Algeria

8 Department of Geography and Regional Planning, University of Oumel Bouaghi, Oum El Bouaghi, Algeria

Published online: 03 June 2023

These effects typical of *Mentha* species have been attributed to polyphenol derivatives and monoterpenoids [6, 7]. Polyphenols are the most important secondary metabolites and bioactive compounds in plants. They are produced via the pentose phosphate, shikimate, and phenylpropanoid pathways [8, 9]. These natural polyphenols have recently gained popularity in the food industry as a safer alternative to artificial molecules such as butylated hydroxyanisole and hydroxytoluene [10]. On the other hand, because of its high phenolic and flavonoid content, *M. rotundifolia* is regarded as a plant with high antioxidant properties [11, 12]. Its aerial part, in particular, contains a variety of flavonoids, including diosmin, naringenin, luteolin, flavonols like kaempferol, and phenolic acids dominated by rosmarinic acid, a phenolic compound thought to be a promising therapeutic agent against a variety of diseases [13, 14].

The study of the effects of various chemical and physical factors, including minerals [15], salt [16], moisture [17], and light [18] on plant growth and the production of secondary metabolites, as well as the mechanisms that promote these metabolites (e.g., culture systems, elicitation, nutrient conditions, removal of toxic products, and precursor feeding, plant hormones), has received a lot of attention due to the pharmacological importance of these secondary plant metabolites. Exogenous phytohormones plant growth regulators (PGRs), in particular, have been found to stimulate plant differentiation, development, and growth even at low concentrations [15–17]. Furthermore, PGRs were suggested to increase specialized metabolite production when applied individually or in combination. For instance, natural auxins including indole acid acetic (IAA), indole-3-butyric acid (IBA), phenylacetic acid (PAA), 1-naphthalene acetic acid (NAA) have been shown to stimulate green alga (*Chlorella vulgaris*) growth and metabolites production [18]. It has also been reported that IAA treatment can stimulate multiple effects on secondary metabolites synthesized in various plants [19, 20]. Furthermore, N6-benzyladeninepurine (BAP) was found to promote the production of phenolic compounds in *Mentha piperita* [21].

The current study sought to examine the impact of IAA and BAP on the content of phenolic compounds in the aerial part and roots of *Mentha rotundifolia* as well as on the plant's bioactivity as measured by antioxidant activity and the inhibitory effect on acetylcholinesterase and glucosidase in light of a large body of evidence demonstrating the effect of PGRs on growth and the production of secondary metabolites in *Mentha* species. The findings of this study will undoubtedly be of great interest because there has been no previous report on this plant in this context.

Material and methods

Chemicals

Acetylcholinesterase (AChE) type VI-S from electriceel < 1000 U/mg solid and butyryl cholinesterase (BChE) from equine serum 100 U/mg protein. α-glucosidase type I from *Saccharomyces cerevisiae* < 10 U/mg solid. 5.5 dithiobis [2-nitrobenzoic acid] (DTNB), butyrylthiocholine chloride, 2.2-diphenyl-1-picrylhydrazyl (DPPH), 2.2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate ($K_2S_2O_8$), linoleic acid, β-carotene, 2.6-di-tert-butyl-4-hydroxytoluene (BHA), tween 40, neocuproine (definition) 6-benzyl amino purine (BAP), and indole acetic acid (IAA) were from Acros organic, Belgium.

Plant material

The collected plants were identified at the species level at the University of Jijel (Algeria), and the experiment was carried out in pot trials using a completely randomized design (CRD). The plants were 8 cm tall when transported on April 15th, 2018, with one plant per pot density. In total, 15 pots, each 26 cm tall and 22 cm in diameter, contained the same soil mixture of clay (55%) and sand (45%). The 15 pots were divided into five groups and each with three replicates. Each group was prepared for treatment with a specific concentration of phytohormone. The physical and chemical characteristics of the soil were determined before the experiment. The parameters pH, electrical conductivity, salinity, solids discounts totals (TDS) were determined using a Multiparameter Pocket Sensor (Palintest) [22]. Chemical analysis was conducted using standard methods including the organic matter and carbon contents [23] total nitrogen evaluated according to the Kjeldahl method [24] assimilable phosphorus content [25] and total calcar ($CaCO_3$) [26]. The results for the soil

Table 1 Physicochemical characteristics of experimentation soil

Parameter	Value
pH	7.72 ± 0.05
Salinity (ppt as NaCl)	41.1 ± 0.01
TDS (ppm)	138 ± 0.5
Conductivity (µS/cm)	0.192 ± 0.9
Organic matter (%)	1.3 ± 0.43
Carbon (%)	0.8 ± 0.28
Nitrogen mg N/100 g	0.49 ± 0.03
Phosphorus (ppm)	2.06 ± 0.56
$CaCO_3$ (%)	41.90 ± 0.86

The results are reported as mean ± SD of 3 different measurements

properties were given in Table 1. It is to note that throughout the experiment, no chemical fertilizer or pesticide was used, and weeds were manually removed; additionally, irrigation was performed at half-field capacity with tap water every day The pots were positioned inside a shelter with a surface area 182.7 m^2 (21.5 m × 8.5 m). The average (minimum–maximum) relative temperature and humidity were 17 and 40 ℃ and 65% and 90%.

Exogenous hormone treatment and sample collection

The hormones BAP and IAA were used at the concentrations of 10 and 20 mg/mL. These concentrations have been chosen based on previous studies reporting the concentrations of these hormones that provide a significant effect on plant development [27–30]. IAA solutions were prepared by dissolving 10 mg and 20 mg of powder in 2 mL of NaOH (1%) in a volumetric flask before dilution with distilled water. On the other hand, the concentration of the BAP solution was prepared by directly dissolving 10 mg and 20 mg in distilled water, adding distilled water to make 1 L, and preparing 10 and 20 ppm solutions, respectively. Plants were sprayed separately with a manual pressure sprayer (50 mL each) with BAP and IAA, concentrating on the leaves and stem until the solution dripped from the tips of the plants. Foliar sprays with different concentrations of IAA and BAP were applied twice (once every 15 days) during the vegetative (July 1) and before flowering (July 15) periods. On August 25, 2018, we harvested the aerial parts and roots after 133 days of cultivation. The plant parts were washed, aerial and root biomass (fresh weight) of plants were measured for each treatment. The plant parts were desiccated for two week and dry weight were measured. Additionally, the plant parts were ground into a fine powder for extraction.

Measurement of total and individual phenolic contents

Preparation of plant extracts

The preparation of the plant extracts was carried out by maceration at room temperature for 24 h with 80% ethanol (80/20, *v/v*) at a solid–liquid ratio of 2.5/50 (*w/v*) under continuous stirring (130 rpm) [31]. The mixture was then filtered through Whatman Millipore No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure (BUCHI, R215, Switzerland). The extracts were placed in small bottles and kept at 4 °C until further analysis.

Total phenolic contents

Total phenol content (TPC) was determined spectrophotometrically by Folin-Ciocalteu as described by Dorman et al. [6] using gallic acid to plot the standard curve. Results were expressed as µg gallic acid equivalents per mg of extract (µg GAE/mg). Total flavonoid content (TFC) was quantified using a spectrophotometric method described by Brahmi et al. based on forming an aluminum-flavonoid complex [32]. Rutin was used as a standard, and the data were expressed in µg of rutin equivalents per mg of extract (µg RE/mg). The amounts of flavones and flavanols (FLC) in aerial parts and roots were determined by using the aluminum-chloride ($ALCl_3$) method reported by Kosalec et al. [33]. Results were expressed as µg quercetin equivalents per milligram of extract (µg QE/mg extract) calculated from the standard curve of quercetin.

Identification of individual phenolic compounds

Identifying individual phenolic compounds was performed using an LC/MS-2020 mass spectrometer (Shimadzu, Kyoto, Japan). The LC system was equipped with an electrospray ionization source (ESI), LC-20AD XR binary pump system, SIL-20AC XR autosampler, CTO-20AC column oven, and a DGU-20AS degasser (Shimadzu, Kyoto, Japan). Spectra were recorded in negative ion mode, monitored, and processed using Shimadzu Lab Solutions LC–MS software. Compounds were separated on an Aquasil C18 column thermostated at 40 °C (150 mm × 3 mm, 3 µm), preceded by an Aquasil C18 guard column (10 mm × 3 mm, 3 µm). The mobile phase comprised phase A (0.1% formic acid in H_2O, *v/v*) and phase B (0.1% formic acid in methanol, *v/v*). The LC-MS conditions were as follows: 10–100% B for 0–45 min, 100% B for 45–55 min with an injection volume of 5 µL and a flow rate of 0.4 mL/min. High-purity nitrogen served as a nebulizer and auxiliary gas. The ion spray voltage was set to − 3.5 V in negative mode. The following settings were applied: a nebulizer gas flow of 1.5 L/min, a dry gas flow of 12 L/min, a DL (dissolution line) temperature of 250 °C, a source block temperature of 400 °C, and a voltage detector of 1.2 V. The results were reported in µg per g of extract.

Antioxidant activity

The antioxidant activity of the extracts was determined using various standard methods. The antiradical capacity was examined using the DPPH method [34], and the results were reported as 50% inhibitory concentration (IC_{50}) in µg/mL. As described by Djermane et al. [35] and Szydowska-Czerniak et al. [36], ferric-reducing power and phenanthroline assays were used to assess the reducing capacity of

iron ions, while cupric reducing antioxidant capacity assay (CUPRAC) was used to measure the reduction copper ions [37]. Furthermore, the antioxidant activity was determined using a β-carotene bleaching assay [34]. Similarly, the results were expressed as $A_{0.5}$ values corresponding to the concentration given an absorbance of 0.5.

Enzyme inhibitory capacity

The acetylcholinesterase inhibition was evaluated by applying Ellman's method with acetylthiocholine as the substrate [38]. The α-glucosidase inhibition was assessed following the protocol described by Lordan et al. [39]. The results were reported using IC_{50} (µg/mL) and inhibition rate (%).

Statistical analysis

The data were first tested for normality and group homogeneity using the QQ plot and Levene's test. A two-way ANOVA analysis was then performed to test for significant differences, with plant organ (aerial part vs. roots) and treatment (T0: control, T1: 10 mg/mL IAA, T2: 20 mg/mL IAA, T3: 10 mg/mL BAP, T4: 20 mg/mL BAP) as factors. In addition, means were separated using Student's Least Significant Difference (LSD) at a 5% significance level.

We used the Pearson correlation coefficient to determine the relationship between phenolic compounds and the biological effects of *M. rotundifolia* (i.e., antioxidant, anticholinesterase, and anti-diabetic activities). Furthermore, principal component analysis (PCA) was used to display treatment localizations based on (1) total phenolic contents and biological activities and (2) individual phenolic compounds.

Results

Effects of IAA and BAP on the fresh and dry weight

The analysis of variance, which included all parts of the plant, showed significant differences between all treatments ($p < 0.01$) for fresh weight and highly significant differences ($p < 0.001$) for dry weight. (Table 2. As shown in Fig. 1, the variation of these parameters strongly depends on the identity of the applied growth factor and its concentration. BAP had the most effect by inducing a two- to three-fold increase (compared to the control) in fresh weight of aerial parts and roots at 10 and 20 mg/mL (Fig. 1A). Similarly, plants grown in 20 mg/mL BAP exhibited a threefold increase in dry weight for the aerial parts. In contrast, the dry weight of the roots was twice as high as in control (T0) (Fig. 1B). IAA treatment resulted in a slight rise in these two parameters in both parts, which was not statistically significant for dry weight.

Table 2 Effect of *M. rotundifolia* plant parts (P, aerial vs. roots) and treatment (T, with 5 levels: 10 mg/mL IAA, 20 mg/mL IAA, 10 mg/mL BAP, 20 mg/mL BAP, and control) and their interaction (P × T) on biomass production and biochemical parameters

	DDL	Frech Weight	Dry Weight	TPC	TFC	FLC	DPPH	CUPRAC	Reducing power	β-Carotene	Phenanthroline	α-Glucosidase	AChE
Effect of (P)	1	57.40***	0.73 ns	1748.95***	16,711.63***	8925.49***	27.85***	2.307**	2920.13***	1,167,392.57***	236.37***	49,189.12***	39,141.79***
Effect of (T)	4	545.79**	59.29***	10,845.57***	145.77***	66.87***	1028.82***	744.38***	821.77***	43,316.35***	185.52***	3065.91***	1426.61***
Effect of (P × T)	4	22.74*	1.78 ns	6211.8***	77.01***	5.21***	266.96***	166.32***	118.89***	46,603.95***	36.16***	1556.55***	822.82***
Residual	20	7.72	3.03		0.997	1.59	0.635	1.189	0.199	1.67	22.27	0.25	0,45	5.46

DDL the degree of freedom, *TPC* total phenolic content, *TFC* total flavonoid content, *FLC* total flavonols content, *DPPH* scavenging capacity of DPPH radical, *CUPRAC* cupric reducing capacity

nsNot significant

**High significance at $p < 0.01$

***Very high significance at $p < 0.001$

Effects of IAA and BAP on biochemical parameters

According to the analysis of variance (Table 2), the interaction part of plant × treatment significantly affected the contents of TPC, TFC, and FLC ($p < 0.001$). The amount of TPC varied from 72.60 ± 1.22 to 229.59 ± 0.13 µg GAE/mg and TFC from 14.16 ± 1.15 to 73.59 ± 0.68 µg RE/mg, while FLC ranged from 8.29 ± 0.78 to 50.68 ± 0.58 µg EQ/mg. When the biochemical parameters were compared between plant parts, the aerial part had greater TFC and FLC than the roots (Table 3). A difference of 271.06% in FLC concentration was found between the aerial part and the roots. However, only a percentage of 9.87% was recorded for TPC (Table 3).

The amount of TPC in the two parts strongly depends on the treatment applied (Fig. 2A). Treatments with 10 mg/mL

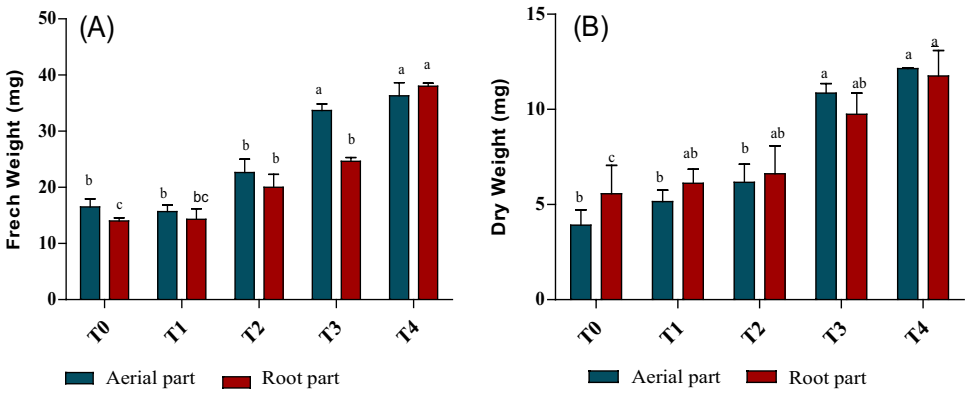

Fig. 1 The effects of plant growth regulators IAA and BAP on aerial parts and roots of *M. rotundifolia* L. **A** Fresh weight and **B** dry weight. T0, T1, T2, T3, and T4 correspond to control, 10 and 20 mg/mL IAA, and 10 and 20 mg/mL BAP, respectively. Column values with different letters are significantly different at $p < 0.05$

Table 3 Mean effect of the plant organ (aerial part vs. roots) on total phenolic content (TPC), total flavonoid content (TFC), and total flavonol content (FLC)

	TPC		TFC		FLC	
	Aerial	Roots	Aerial	Roots	Aerial	Roots
Means (± SD)	169.94 ± 43.6[a]	154.67 ± 54.5[b]	67.29 ± 6.3[a]	20.08 ± 5.1[b]	47.22 ± 3.7[a]	12.73 ± 2.7[b]
Accumulation (%)[a]	9.87		235.06		271.06	
$LSD_{5\%}$	0.76		0.961		0.606	

Shown are the homogeneous groups and percentage of accumulation in the aerial part compared to the roots, which was obtained using the following formula % accumulation = ((VAP − VRP))/VRP × 100), where VAP corresponds to the value of the variable (TPC, TFC, or FLC) accumulated in the extract' aerial part and VRP to the value accumulated in the root part of the same extract. Values with different letters are significantly different at $p < 0.05$

$LSD_{5\%}$ refers to *Least Significant difference*

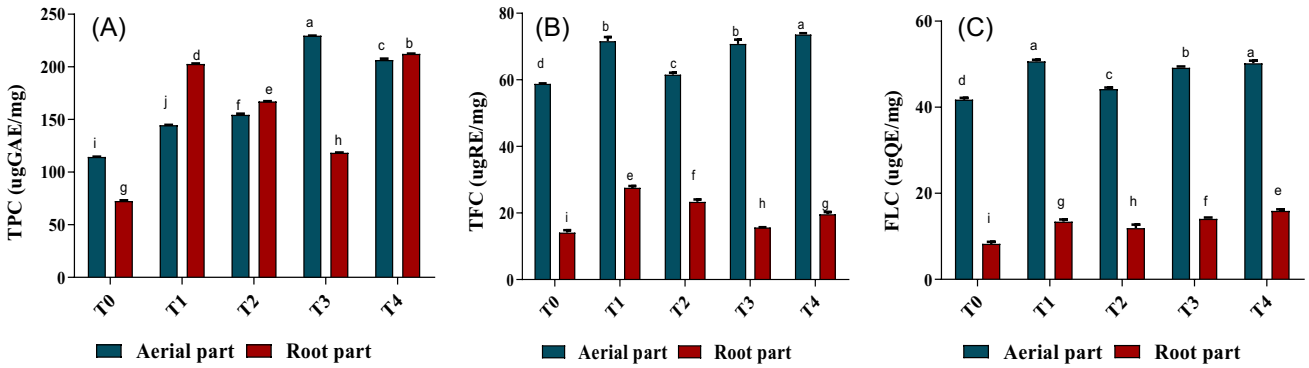

Fig. 2 Effect of plant growth regulators IAA and BAP on **A** total phenolics contents (TPC), **B** total flavonoids contents (TFC), and **C** flavonols contents (FLC) in the aerial parts and roots of *M. rotundifolia*. T1, T2, T3, and T4 correspond to 10 and 20 mg/mL IAA and 10 and 20 mg/mL BAP, respectively. T0: correspond to the untreated samples (Control). Column values with different letters are significantly different at $p < 0.05$

BAP and 20 mg/mL BAP (T3 and T4, respectively) resulted in the highest TPC values in the aerial parts of 229.59 ± 0.13 and 206.64 ± 1.88 µg GAE/mg, respectively. When 20 mg/mL of BAP (T4) was applied to the roots, the maximum TPC was 212.3 ± 0.4 µg GAE/mg compared to 202.89 ± 0.72 µg GAE/mg obtained under 10 mg/mL of IAA (T1).

The amounts of TFC and FLC also depended on the treatment and the part of the plant (Fig. 2B, C). Treatment with 10 mg/mL IAA (T1) and 20 mg/mL BAP(T4) produced the highest values of TFC (71.62 ± 2.12 and 73.59 ± 0.68 µg RE /mg, respectively) and FLC (50.68 ± 0.58, 50.23 ± 0.99 µg QE/mg) in aerial part. About the roots, 10 mg/mL of IAA (T1) gave the highest content of TFC (27.61 ± 0.89 µg RE/mg), followed by 20 mg/mL of IAA (T2) with a value of 23.34 ± 1.27 µg RE/mg, then by 20 mg/mL BAP (T4) with a value of 19.64 ± 1.13 µg RE/mg, while the highest amount of FLC (15.92 ± 0.63 µg QE/mg) was obtained under 20 mg/mL BAP (T4) treatment. These observations indicate that BAP increased TPC in aerial parts and roots, while IAA significantly enhanced TFC in roots of *M. rotundifolia*.

Effects of IAA and BAP on phenolic profile in aerial part and roots of *M. rotundifolia*

Seventeen compounds were identified, including phenolic acids and flavonoids in the aerial part and root extracts. Depending on the phenolic compounds, the kind (IAA or BAP) and amount (10 or 20 mg/mL) of growth factor, and plant part (aerial vs. roots), treatments with growth regulators resulted in considerable increases in the level of phenolic compounds compared to the control. Among the phenolic compounds studied, quinic acid, rosmarinic acid, and salviolinic acid were identified as the main phenolic compounds in the aerial part with amounts of 59.54, 62.57, and 73.65 µg/g, respectively (Table 4). The main phenolics in the roots were salviolinic (41.32 µg/g), rosmarinic (32.13 µg/g), quinic (17.10 µg/g), and caffeic (14.40 µg/g) acids, which together made up the majority of the phenolic compounds in the roots (Table 4).

The aerial part appeared to respond better to treatment with BAP, which generally had the most notable impact on the quantities of phenolic compounds. The amount of salviolinic acid rose from 10.44 (T0) to 73.65 µg/g in the aerial part receiving 20 mg/mL of BAP (T4), showing the largest increase. Additionally, the amount of rosmarinic acid in the aerial component dramatically increased with the T3 and T4 treatments (10 and 20 mg/mL of BAP), increasing it from 17.34 to 66.27 and 62.57 µg/g, respectively. On the other hand, trans-ferulic acid was not found in the aerial part, but after stimulation by growth factors, amounts ranging from 0.03 to 0.06 µg/g were found. The quantity of salvianolic acid in the roots raised the most with the T3 treatment

Table 4 Phenolic components in the aerial part and roots of *M. rotundifolia* treated with indole acid acetic (IAA) and N6-benzyladeninepurine (BAP)

	Tr	Aerial part						Roots			
		IAA			BAP			IAA		BAP	
		T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
Quinic acid	2.474	35.523	37.042	42.548	59.535	46.249	9.343	8.906	9.648	17.104	15.906
Gallic acid	2.258	ND	ND	ND	ND	ND	0.221	0.441	1.002	0.487	0.182
Protocachuic acid	2.258	2.472	6.291	1.292	1.834	1.202	4.998	7.050	5.453	8.754	6.004
Caffeic acid	7.016	1.689	14.813	2.754	1.883	3.351	12.517	11.130	10.843	14.397	10.013
Syringic acid	15.989	3.384	6.131	3.032	3.321	2.463	4.618	5.547	3.128	6.714	3.751
p-Coumaric acid	18.094	0.692	1.394	0.197	0.188	0.207	0.028	0.027	0.014	0.063	0.041
Trans-ferulic acid	25.551	ND	0.058	0.029	0.031	0.030	0.043	0.040	0.033	0.058	0.038
Rutin	22.801	0.253	0.147	0.341	0.409	0.729	0.055	0.077	0.083	0.059	0.097
Luteolin-7-*o*-glucoside	25.510	1.449	0.147	1.718	2.693	3.065	0.034	0.014	0.032	0.041	0.035
Naringin	27.863	1.749	0.866	1.119	1.431	1.891	ND	ND	ND	ND	ND
Apegenin-7-*o*-glucoside	28.076	0.466	0.180	0.537	0.575	0.695	0.023	0.026	0.011	0.033	0.022
Rosmarinic acid	30.823	17.336	25.944	42.509	66.265	62.566	30.979	23.965	28.618	31.703	32.132
Salvianolic acid	30.011	10.436	36.595	20.218	37.46	73.651	18.232	20.810	23.283	41.317	25.962
Naringenin	31.220	0.320	0.611	0.819	0.608	0.713	1.738	1.527	5.931	1.475	2.432
Apigenin	37.027	0.186	0.586	0.430	0.273	0.399	0.126	0.098	0.401	0.122	0.482
Cirsiliol	37.578	0.079	1.037	1.119	1.966	1.987	0.650	0.669	0.546	1.120	0.767

Rt retention time, *ND* not detected

Values represent the quantities of individual compounds in µg/g of extract. T0, control; T1, 10 mg/mL IAA; T2, 20 mg/mL IAA; T3, 10 mg/mL BAP; T4, 20 mg/mL BAP

(10 mg/g), going from 18.23 to 41.32 µg/g, whereas just a modest rise was seen for the other treatments.

Effects of IAA and BAP on antioxidant activity

Antioxidant activity has been significantly influenced by treatment, plant part, and interaction ($p < 0.001$) (Table 2). The results also demonstrated that the extracts had variable activity levels, as shown by the IC_{50} and $A_{0.5}$ values (Fig. 3). Lower IC_{50} and $A_{0.5}$ values in this figure imply stronger antioxidant activity. The kind of phytohormone, its concentration, and the part of the plant all affected how the DPPH antiradical effect varied. The aerial part was generally more effective than the roots in DPPH scavenging (Fig. 3A). Compared to the control ($IC_{50} = 42.42 \pm 1.46$), T1 treatment had the highest antioxidant activity in the aerial part ($IC_{50} = 8.41$ µg/mL). For the roots, however, the T2 treatment had the highest antioxidant activity with an IC_{50} value of 11.88 ± 1.39 µg/mL (compared to the control: $IC_{50} = 49.69 \pm 1.45$ µg/mL). Overall, the effect of the treatment on the reduction of DPPH radical in the aerial part and roots can be presented in the following decreasing orders, respectively: T1 > T3 > T4 > T2 > T0, and T2 > T1 > T4 > T3 > T0 (Fig. 3A).

Figure 3B illustrates how the various treatments reduced the copper ions. Compared to the control, T4 in the aerial part and T2 in the roots were shown to have the greatest capacities ($A_{0.5} = 10.58 \pm 0.36$ and 14.48 ± 0.26 µg/mL, respectively). The other treatments reduced copper ions with moderate to good activity; the $A_{0.5}$ values for T2, T3, and T1 in the aerial part were 15.62 ± 0.54, 20.93 ± 0.84 µg/mL and 31.07 ± 0.08 µg/mL, respectively. While values of 22.21 ± 0.11, 23.15 ± 0.12, and 28.58 ± 0.37 µg/mL were

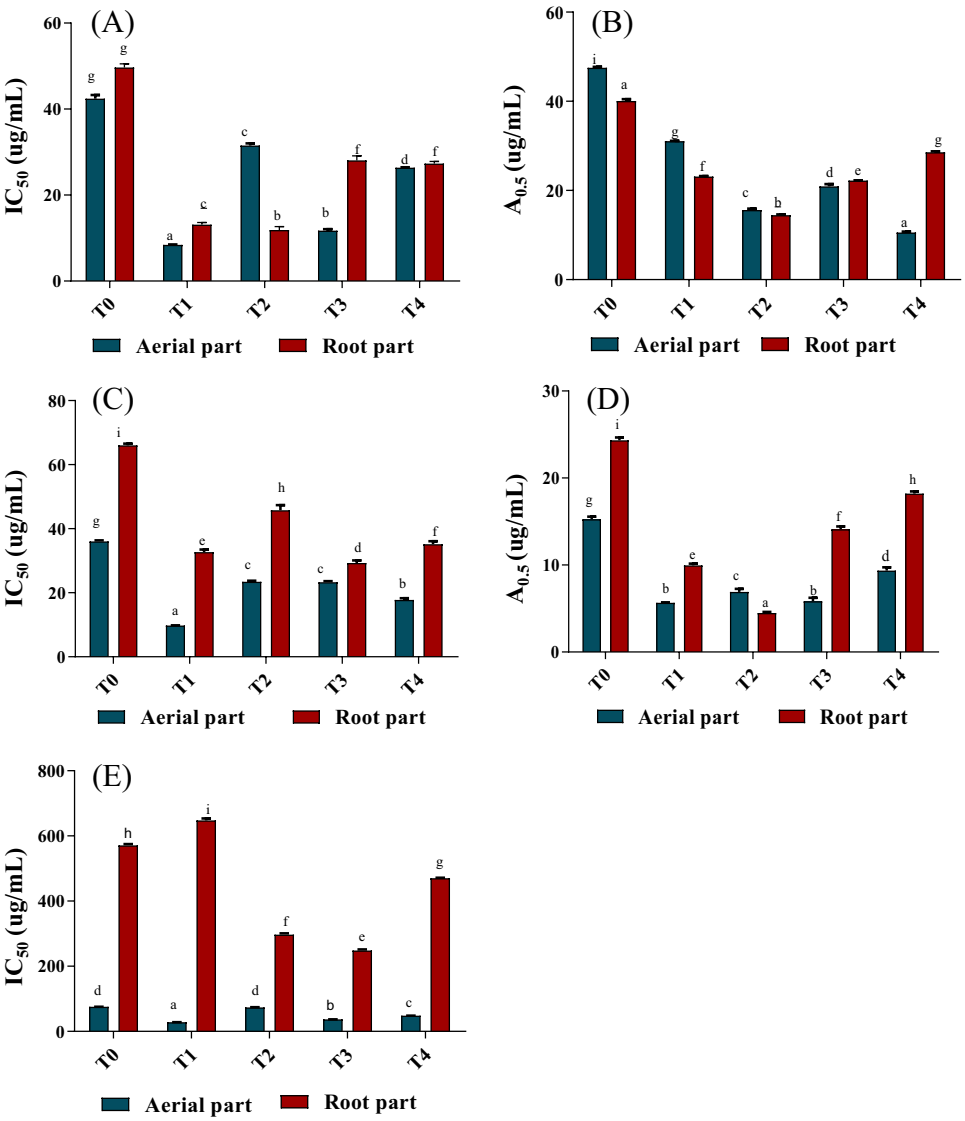

Fig. 3 Antioxidant activities of M. *rotundifolia* treated by different concentrations of IAA and BAP. **A** DPPH, **B** cupric-reducing antioxidant capacity (CUPRAC), **C** ferric-reducing power, **D** phenanthroline, and **E** β-carotene assays were used. T1, T2, T3, and T4 correspond to 10 and 20 mg/mL IAA and 10 and 20 mg/mL BAP, respectively. T0: correspond to the untreated samples (Control). Column values with different letters are significantly different at $p < 0.05$

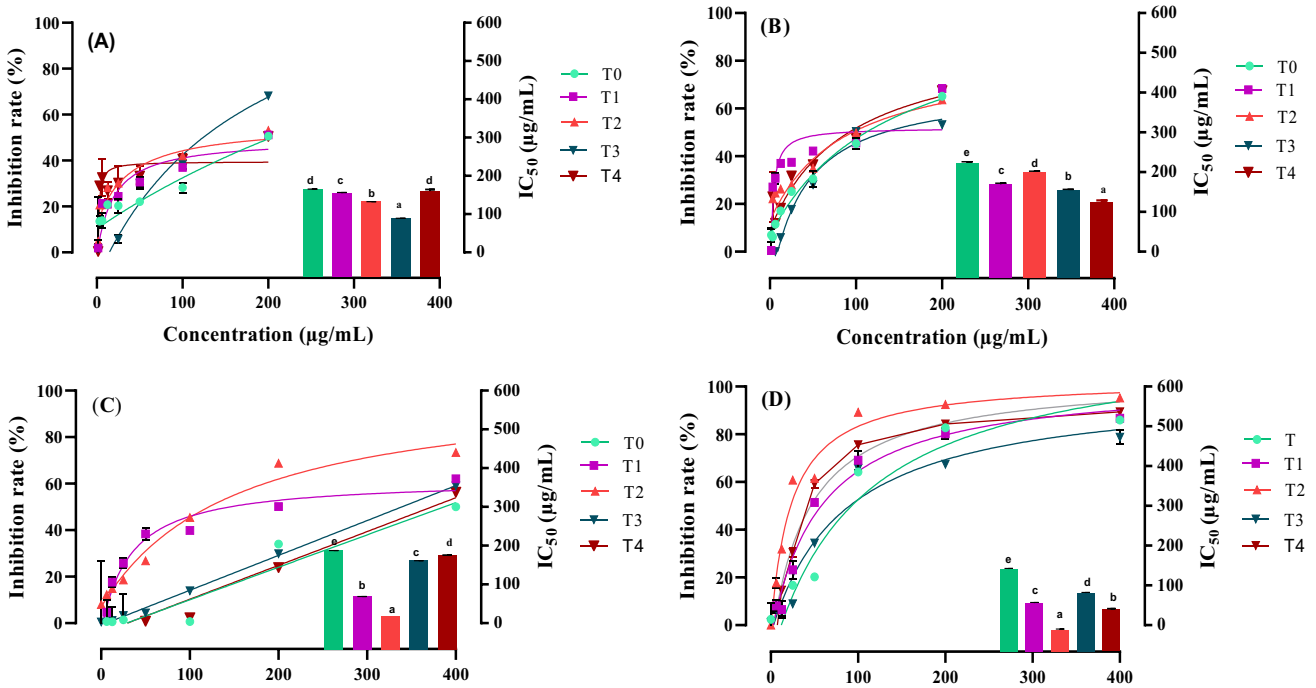

Fig. 4 Inhibitory rate and IC_{50} values of *M. rotundifolia* extracts under different conditions. **A, B** show AChE inhibitory assay for the aerial part and roots, respectively. **C**, **D** show α-glucosidase assay for aerial part and roots, respectively. T1 and T2 correspond to treatments with 10 and 20 mg/mL of IAA, whereas T3 and T4 represent treatments with 10 and 20 mg/L BAP. T0 refers to untreated samples (Control). Column values with different letters are significantly different at $p < 0.05$

found in the roots of samples treated with T3, T1, and T4, respectively.

All extracts showed a significant capacity to decrease iron ions (Fig. 3C). The ferric-reducing power diminishes in the aerial part in the following order: T1 > T4 > T3 > T2 > T0, and in the roots, T3 > T4 > T1 > T2 > T0 (Fig. 3C). The ferric-reducing capacity of the aerial part ($A_{0.5}$ values ranging from 9.77 ± 0.15 to 36.02 ± 0.61 µg/mL) was significantly higher than that of the roots (Fig. 3C). The phenanthroline method for reducing iron ions also revealed remarkable reducing capacities in all extracts (Fig. 3D). Compared to the control, growth regulator treatments significantly increased the reducing capacity. Except for the T2 treatment in the roots, which indicated the lowest $A_{0.5}$ (4.48 ± 0.13 µg/mL), the aerial part showed a significantly better effect than the roots. The $A_{0.5}$ values changed between 5.68 ± 0.04 and 9.37 ± 0.46 µg/mL within the aerial part in comparison to the control (15.25 ± 0.41 µg/mL). Though they shifted between 4.48 ± 0.13 µg/mL and 18.22 ± 0.30 µg/mL in the roots compared to the control ($A_{0.5}$ = 24.35 ± 0.39 µg/mL) (Fig. 3D).

The results of the antioxidant activity examined by the β-carotene/linoleic acid system showed that the aerial part remarkably inhibited the bleaching of β-carotene. The treatments significantly favored this activity compared to the control (Fig. 3E). T1 had the most significant impact in the aerial part (IC_{50} = 28.18 ± 0.51 µg/mL), followed by T3 (IC_{50} = 36.89 ± 0.51 µg/mL), T4 (IC_{50} = 48.08 ± 0.47 µg/mL), and lastly, T2 (IC_{50} = 73.65 ± 0.64 µg/mL), which had the same effect as the control (IC_{50} = 75.56 ± 0.55 µg/mL). Remarkably, while T2, T3, and T4 treatments increased root activity, T1 decreased it (IC_{50} = 647.47 ± 10.70 g/mL) compared to the control (IC_{50} = 571.31 ± 5.95 µg/mL) (Fig. 3E).

Influences of IAA and BAP on acetylcholinesterase (AChE) and α-glucosidase inhibitory effects

According to the analysis of variance, all the factors significantly affect the AChE and α-glucosidase inhibitory activity ($p < 0.001$) (Table 2). However, treatments with IAA and BAP led to slight increases in the inhibitory effect of AChE and α-glucosidase (Fig. 4). The roots impact both enzymes better than the aerial parts (Fig. 4). Compared to IAA, BAP treatments had the highest effect against AChE in both plant organs (Fig. 4A, B). The most potent inhibitory capacity against AChE in the aerial parts was observed with T3 (IC_{50} = 146.39 ± 4.24 µg/mL) followed by T2 (IC_{50} = 172.87 ± 0.86 µg/mL), while the control showed weak inhibitory activity (IC_{50} = 199.60 ± 2.05 µg/mL) (Fig. 4A). T3 and T4 treatments displayed greater inhibitory capabilities for the roots, with IC_{50} values of 99.66 ± 0.43 µg/mL and 85.75 ± 2.80 µg/mL, respectively, as compared to the control (IC_{50} = 129.89 ± 1.94 µg/mL). In

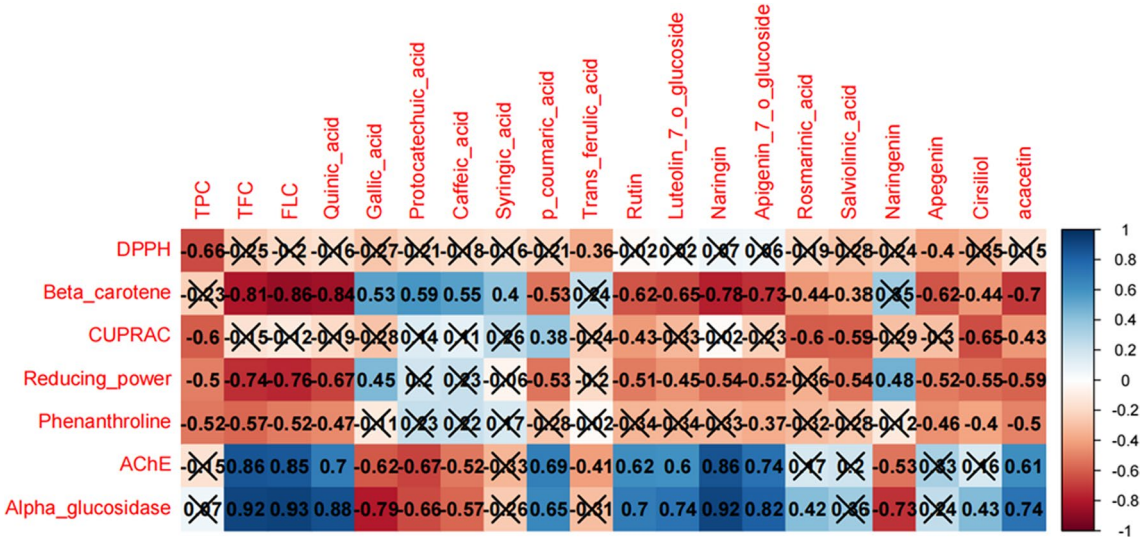

Fig. 5 Pearson's correlation coefficients among biological activities and total and individual phenolic components of *M. rotundifolia* extracts. Values ticked with an "X" are statistically non-significant

contrast, T1 and T2 treatments had poor inhibitory capabilities, with IC_{50} values of 105.43 ± 2.18 and 119.55 ± 1.33 µg/mL, respectively.

The highest inhibitory effect on α-glucosidase was observed with T2 in the roots (IC_{50} = 22.28 ± 0.57 µg/mL) (Fig. 4D). Compared to the control (IC_{50} = 83.24 ± 0.27 µg/mL), T4, T1, and T3 treatments also induced remarkable increases in inhibitory activity in roots (IC_{50} values of 41.12 ± 1.15, 48.50 ± 1.05, and 58.92 ± 0.58 µg/mL, respectively). Except for T2 treatment, which gave a remarkable effect (IC_{50} = 127.00 ± 0.24 µg/mL), most of the extracts of the aerial part showed a weak α-glucosidase inhibitory activity with IC_{50} > 200 µg/mL in all cases (Fig. 4C).

The relationship between biological activities and total and individual phenolic compounds

The correlation coefficients between measures evaluating antioxidant activity and TPC, TFC, and FLC ranged from − 0.517 to − 0.864 ($p < 0.05$). TPC was correlated with DPPH (r = − 0.66), CUPRAC (r = − 0.60), ferric-reducing power (r = − 0.50), and phenanthroline (r = − 0.52). TFC and FLC showed strong relationships with ferric-reducing power (r = − 0.74 and r = − 0.76, respectively), β-carotene bleaching activity (r = − 0.81 and − 0.86, respectively), and moderate relationships with phenanthroline (r = − 0.57 and − 0.52, respectively) (Fig. 5).

The correlation analysis of individual phenolic compounds revealed that rosmarinic acid, salviolinic acid, and cirsiliol were negatively connected with the CUPRAC assay (Fig. 6). Quinic, *p*-coumaric, and salvianolic acids and rutin, apigenin, and acacetin were associated with the iron-reducing activity. Ferric-reducing power was also negatively correlated with quinic acid, *p*-coumaric acid, salviolinic acid, rutin, luteolin-7-o-glucoside, naringin, apegenin-7-*O*-glucoside, apigenin, cirsiliol, and acacetin. β-Carotene assay was negatively correlated with the following phenolic compounds: quinic acid, *p*-coumaric-acid, rutin, luteolin-7-*o*-glucoside, naringin, apigenin-7-*O*-glucoside, rosmarinic acid, salviolinic acid, apigenin, cirsiliol, and acacetin. Furthermore, the phenanthroline assay showed negative associations with quinic acid, apigenin-7-*O*-glucoside, apigenin, cirsiliol, and acacetin (Fig. 6). Regarding inhibitory activity, AChE, and α-glucosidase were significantly and negatively correlated with gallic acid, protocatechuic acid, caffeic acid, and naringenin. In contrast, they were significantly and positively correlated with quinic acid, *p*-coumaric acid, rutin, luteolin-7-O-glucoside, naringin, apigenin-7-*O*-glucoside, and acacetin.

According to the first PCA biplot (Fig. 6A), T1, T2, T3, and T4 had the highest TFC, FLC, and inhibitory activity (AChE and α-glucosidase) in the aerial part. On the other hand, T1, T2, T3, and T4 roots had the highest levels of β-carotene assay. The second PCA biplot (Fig. 6B) showed that in the aerial part, T1 had high levels of trans-ferric acid, while T2, T3, and T4 had high levels of luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, rosmarinic and quinic acids, acacetin, rutin, and luteolin-7-*O*-glucoside. T3 roots contained high levels of syringic, protocatechuic, and caffeic acids, whereas T1, T2, and T4 roots contained higher levels of gallic acid and naringenin.

Fig. 6 Biplots show the gathering of treatments based on **A** total phenolic compounds and biological activities and **B** individual phenolic compounds in the aerial part and roots of treated and untreated *M. rotundifolia* extracts

Discussion

Several articles have reported the ability to stimulate the accumulation of phytochemicals using PGRs in vivo and in vitro of many species of the Lamiaceae family [40–43]. Accordingly, the effects of IAA and BAP on the synthesis of polyphenolic compounds in *M. rotundifolia* and its biological activity has been investigated through the present work.

The plant weight (fresh and dry) significantly increased after treatment with IAA and BAP.. similarly, the treatment of *Guizotia abyssinica* (L.f.) Cass with IAA and BAP-significantly increased the aboveground biomass [44]. Also BAP spraying led to an optimal biomass production in *Thymus*

leucotrichus Hal. Shoots [45], and a three-fold increase in *M. piperita*'s root dry weight was observed [16]. In particular, the application of 10 mg/L of BAP clearly increases the fresh weight and plant height of *Phellodendron* Chinese seedlings. [29]. The authors of these studies hypothesized that BAP might play a role in controlling cell division, shoot and root development, apical dominance, and the development of lateral buds, Indeed, exogenous BAP plays a key regulatory role in the growth of different plants, which could explain the observed results [16, 46].

Our study showed that, compared with untreated plants, all PGRs treatments contributed to a higher TPC, TFC, and FLC. In general, although the effects of auxin and cytokinin on polyphenols synthesis are not well understood, it is common that a single plant hormone can regulate a wide range of physiological and growth processes [16]. However, the underlying mechanism of BAP regulating plant secondary metabolic biosynthesis pathway has yet to be elucidated, but it has been proven that kinetin (cytokinin type), for example, can incorporate into tRNA, influencing therefore amino acid metabolism and protein synthesis and it is to be suggested that by affecting the gene expression of key enzymes during the plant development process, the production of polyphenols could be affected [47]. Also, auxins trigger a signal transduction pathway that produces secondary messengers that immediately activate pre-existing H^+-ATPases and boost the expression of several genes involved in growth and development. A specially, IAA plays a key role in cell division, elongation, differentiation and in regulating the interaction with the environment [48]. Shah et al. [47] recorded that polyphenol synthesis was initiated after the cells embarked upon the phase of most rapid growth in Cassia tissues cultured in vitro. It makes sense to assume that by promoting cell proliferation in a certain hormonal milieu that encourages the derivative cells for enhanced polyphenol production, it may be impacting polyphenol production [37].

Overall, the effect of IAA and BAP has been widely studied in vitro cultures and have reported that various secondary metabolites can be stimulated by affecting metabolic pathways and increasing sensitivity to endogenous PGRs, resulting in high variability and several unpredictable events [49]. For example, in hairy root crops of the *Tartarian buckwheat* cultivar, IAA increased anthocyanin production [17]. Similarly, the level of phenolic compounds was increased in the hairy roots of *Panax ginseng* C. A. Meyer grown on a medium supplemented with a low concentration of IAA [50]. Also, BAP enhanced total phenolics and flavonoids in *Thymus vulgaris* and *Origanum vulgare* and improved total phenolics in the aerial parts of *M. piperita* [21, 51], according to Jessica et al. [52] *Amburana cearensis* (cumar) extracts treated with BAP during in vitro cultivation show higher flavonoid component levels than extracts from plants produced under conventional conditions.

It is suggested that the production of polyphenols could affect by the gene expression of key enzymes during the plant development process [47]. For example, anthocyanin accumulation in *Arabidopsis thaliana* is increased by exposure to benzyl adenine (BA), which induces the expression of phenylalanine ammonia-lyase, a key enzyme in cinnamate biosynthesis [53]. Although the role of IAA or BAP in the phytochemical's biosynthesis pathway need further studies in order to decipher the somewhat complex interactions but there has been direct evidence for the influence of IAA and BAP on polyphenols accumulation in field-grown plant. For instance, Li et al. [54] have reported the ability of IAA to enhance flavonoid accumulation in field-grown *Glycyrrhiza uralensis* significantly.. In another study [48], it was declared that exogenous application of IAA significantly increased phenol content in *Hibiscus sabdariffa* L., confirming the results of the present work. Besides, the hormone BAP has been reported to significantly enhance the anthocyanin content of grape fruits and the expression levels of F3GT, CHS, and PAL in 90 days after harvest [55]. Also, It was reported that foliar application of BAP induced the expression of the MaF3GT, Ma4CL, MaPAL and MaCHS genes, which regulate flavanol synthesis [56]. Indeed, PAL, CHS, 4CL and F3GT are considered as the key enzymes in flavonoids synthesis pathway [57, 58]. In this study, IAA and BAP treatments significantly increased salvianolic, rosmarinic, and quinic acids, among other phenolic compounds. These findings are consistent with the literature on using growth factors to stimulate secondary metabolite production in cell cultures. Weremczuk-Jeyna et al. found an increase in salvianolic and rosmarnic acids production in *Dracocephalum forrestii* shoots grown in a medium supplemented with varying concentrations of IAA and BAP [59]. Grzegorczyk-Krolak et al. also observed stimulation of flavone production (baicalin, wogonoside, luteolin, luteolin-7-*O*-glucoside, verbascoside) in *Scutellaria altissima* shoot cultures supplemented with various growth factors, including BAP [43]. Moreover, the application of 30 mg/L of BAP significantly improved the contents of rutin, isoquercitrin, and astragalin contents, and it dramatically promoted the expression of genes involved to flavonoids production [56]. Indeed, investigations have shown that BAP promotes the accumulation of bioactive compounds at certain amounts. For example, *Hellodendron chinense* seedlings treated with 10 or 20 mg/L BAP significantly exhibited greater amounts of berberine and phellodendrine [29]. According to Taha et al. growth factors influence the biosynthesis by regulating the expression of enzymes involved in biosynthetic reactions and by controlling the rate of expressed proteins [60].

Despite the antioxidant activity of the naturally grown *M. rotundifolia* being extensively reported [61, 62], this is the first report on the effect of PGRs on antioxidative activities in the aerial parts and roots of this species using different

methods. Our results showed that when the extracts were exposed to different concentrations of IAA and BAP, their antioxidant effect was significantly enhanced, consistent with previous studies that confirmed the effect of growth regulators on the antioxidant properties of treated plants. In vitro cultivated *M. piperia*, for example, was observed to increase inhibition of linoleic acid peroxidation and decrease hydroperoxide production when treated with BAP [21]. Furthermore, *S. alpina* shoots grown in the presence of benzyl adenine and auxin were reported to produce a significant effect on free radical scavenging and the ferric-reducing ability.

The enhancement of antioxidant activity observed in this study is primarily due to the composition of the extracts, specifically the presence of polyphenolic compounds, which are considered powerful natural antioxidants. Flavonoids, in particular, are a class of polyphenols known for their various pharmacological effects on the human body; they have been identified as potent antioxidants, which explains the significant antioxidant effect of *M. rotundifolia* extracts. Furthermore, the observed variability in antioxidant activity could be attributed to differences in polyphenol accumulation among the samples and the assay's mechanism of action. This result is confirmed by the correlation analysis showing a moderate to strong correlation between the antioxidant capacity and the TPC, TFC, and FLC. Overall, many other studies have observed such kinds of relationships between the antioxidant activity of plant extracts and their polyphenolic contents [63–65].

Correlation analysis also revealed moderate correlations between certain individual phenolic compounds and the various antioxidant tests used. It has been argued that individual phenolic compounds can exert their antioxidant effect independently or interact with each other, producing a synergistic or antagonistic effect [66]. These compounds can neutralize free radicals and act as reductones by donating electrons to regenerate an oxidized molecule or stop the chain reaction of free radicals [67]. Furthermore, the variation in the level of different individual compounds can directly influence antioxidant activity depending on their mechanism of action [68].

Plants exposed to PGRs had significantly greater inhibitory capacity than untreated plants. In contrast to the antioxidant effect, where the aerial parts were found to be more potent than the roots, the roots were more effective in inhibiting acetylcholinesterase and α-glucosidase despite their low polyphenol content, indicating the presence and contribution of other compounds to the activity. In particular, a concentration of 20 mg/L BAP induced the most substantial effect on root acetylcholinesterase, which is consistent with the literature stating that the type of PGR can determine the quality and quantity of synthesized metabolites [49]. Some authors have linked the anticholinesterase effect of *M. rotundifolia* to the presence of specific compounds such as luteolin, hesperidin, and salvianolic acid [69, 70]. Others have also reported the influence of auxins and cytokinins on the accumulation of rosmarinic acid in *Coleus blumei* [71], which is associated with the presence of other flavonoids that contribute to the inhibition of acetylcholinesterase [72, 73]. Moreover, consistent with previous reports [74], this study showed significant relationships between AChE inhibitory activity and concentrations of caffeic acid and naringenin.

The α-glucosidase is a key enzyme in the degradation of dietary carbohydrates and is the main target of hypoglycemic drugs in managing type 2 diabetes. In this study, roots showed excellent anti-diabetic activity compared to the aerial parts indicating that the nature of the phenolic compounds or the presence of other phytochemicals strongly determines this activity. Polyphenols are known for their ability to bind to proteins and carbohydrates through multiple hydrogen bonds and hydrophobic associations resulting in the blocking of catalytic sites of enzymes [75]. Finally, few naturally grown *Mentha* species have been tested for α-glycosidase inhibition, including *M. rotundifolia, M. spicata* and *M. longifolia*, which have shown excellent potential in polyphenol and flavonoid accumulation [31, 76, 77]. However, to the best of our knowledge, this is the first report on the effect of PGRs on the anti-diabetic activity of *M. rotundifolia* by measuring their abilities to inhibit α-glucosidase.

Conclusions

The current study examined the effect of the growth regulators IAA and BAP on the accumulation of phenolic compounds in *M. rotundifolia* and consequently on its bioactivity. The contents of phenolic compounds significantly increased after treatment with growth regulators in the aerial parts and roots. In addition, an increase in individual phenolics was mainly observed for salvianolic and rosmarinic acids in the aerial parts treated with 20 or 10 mg/mL of BAP. Correlations between phenolic contents or individual phenolics and the bioactivity of extracts was observed. More particularly, the treatment with the exogeneous hormones promoted the antioxidant activity and the anti-α-glucosidase ability of the roots. These results can lead to two main conclusions: first, *M. rotundifolia* may serve as a potent source of bioactive compounds with a variety of biological effects, and second, the results may provide a basis for the treatability of naturally grown plants to improve their phytochemical yield and their bioactivity which could constitute an economic interest for their exploitation. However, further genetic studies are needed to identify the underlying roles of cytokinins and auxins in polyphenols accumulation and clarify the regulatory mechanism by which IAA and BAP affect the polyphenols synthesis.

Acknowledgements The authors thank D[r]. Bensouici Chawki (Biotechnology Research Center, Constantine, Algeria) for his friendly support. Thanks also go to DGRSDT for financial support.

Data Availability The data used to support the findings of this study are available upon request. Interested researchers can contact [corresponding author's email address (h.kecies@centre-univ-mila.dz)] to obtain access to the data.

References

1. B.M. Lange, R. Croteau, Curr. Opin. Plant Biol. **2**, 139 (1999)
2. M. Sevindik, J. Tradit. Med. Clin. Nat. **7**, 259 (2018)
3. R. Yakoubi, S. Megateli, T.H. Sadok, L. Gali, Biocatal. Agric. Biotechnol. **34**, 102038 (2021)
4. L. Moreno, R. Bello, E. Primo-Yúfera, J. Esplugues, Phyther. Res. **16**, 10 (2002)
5. S. Ladjel, N. Gherraf, D. Hamada, J. Appl. Sci. Res. **7**, 1665 (2011)
6. H.J.D. Dorman, M. Koşar, K. Kahlos, Y. Holm, R. Hiltunen, J. Agric. Food Chem. **51**, 4563 (2003)
7. M. Brada, M. Bezzina, M. Marlier, A. Carlier, G. Lognay, Biotechnol. Agron. Soc. Environ. **11**, 3 (2007)
8. D.-O. Kim, S.W. Jeong, C.Y. Lee, Food Chem. **81**, 321 (2003)
9. R. Randhir, Y.-T. Lin, K. Shetty, Y.-T. Lin, Asia Pac. J. Clin. Nutr. **13**(3), 295 (2004)
10. S.R. Kanatt, R. Chander, A. Sharma, Food Chem. **100**, 451 (2007)
11. L. Riahi, M. Elferchichi, H. Ghazghazi, J. Jebali, S. Ziadi, C. Aouadhi, H. Chograni, Y. Zaouali, N. Zoghlami, A. Mliki, Ind. Crops Prod. **49**, 883 (2013)
12. F. Brahmi, T. Nury, M. Debbabi, S. Hadj-Ahmed, A. Zarrouk, M. Prost, K. Madani, L. Boulekbache-Makhlouf, G. Lizard, Antioxidants **7**, 184 (2018)
13. F. Siham, B. Rachid, R.M. Al-Zoubi, Pharmacogn. J. **11**(3), 521 (2019)
14. M. Nadeem, M. Imran, T. Aslam Gondal, A. Imran, M. Shahbaz, R. Muhammad Amir, M. Wasim Sajid, T. Batool Qaisrani, M. Atif, G. Hussain, Appl. Sci. **9**, 3139 (2019)
15. T. Gaspar, C. Kevers, C. Penel, H. Greppin, D.M. Reid, T.A. Thorpe, In Vitro Cell. Dev. Biol. **32**, 272 (1996)
16. M.V. Santoro, F. Nievas, J. Zygadlo, W. Giordano, E. Banchio, Am. J. Plant Sci. **04**, 49 (2013)
17. C.H. Park, A. AyeThwe, S.J. Kim, J.S. Park, M. Arasu, N.A. Al-Dhabi, N. Il Park, S.U. Park, Nat. Prod. Commun. (2016). https://doi.org/10.1177/1934578X1601100924
18. A. Piotrowska-Niczyporuk, A. Bajguz, Plant Growth Regul. **73**, 57 (2014)
19. M.J.C. Rhodes, A.J. Parr, A. Giulietti, E.L.H. Aird, in *Primary and Secondary Metabolism of Plants Cell Cultures III* (Springer, Berlin, 1994), pp. 143–151.
20. R.R.J. Arroo, A. Develi, H. Meijers, E. Van de Westerlo, A.K. Kemp, A.F. Croes, G.J. Wullems, Physiol. Plant. **93**, 233 (1995)
21. A. Parić, E. Karalija, J. Čakar, Acta Biol. Szeged. **61**, 189 (2017)
22. J. Rodier, B. Legube, N. Merlet, Entièrement Mise À Jour Dunod Paris (2009)
23. A. Walkley, I.A. Black, Soil Sci. **37**, 29 (1934)
24. J.A. Parkinson, S.E. Allen, Commun. Soil Sci. Plant Anal. **6**, 1 (1975)
25. S.R. Olsen, L.E. Sommers, *Methods of Soil Analysis*, vol. 581 (Soil Science Society of America, Madison, 1982)
26. D. Soltner, *Les bases de la production végétale - Tome I*, Paris (2005)
27. A. di Benedetto, C. Galmarini, J. Tognetti, J. Hortic. Sci. Biotechnol. **88**, 179 (2013)
28. A. Benedetto, C. Galmarini, J. Tognetti, Am. J. Exp. Agric. **5**, 419 (2014)
29. H. He, J. Qin, X. Cheng, K. Xu, L. Teng, D. Zhang, Saudi. J. Biol. Sci. **25**, 1189 (2018)
30. D. Nassef, H. El-aref, Egypt. J. Hortic. **45**, 121 (2018)
31. H. Kecis, Y. Abdelouahab, M. Bagues, L. Gali, Biocatal. Agric. Biotechnol. **47**, 102581 (2023)
32. B. Fatiha, H. Didier, G. Naima, M. Khodir, K. Martin, K. Léocadie, S. Caroline, C. Mohamed, D. Pierre, Ind. Crops Prod. **74**, 722 (2015)
33. I. Kosalec, M. Bakmaz, S. Pepeljnjak, S. Vladimir-Knezevic, Acta Pharm. **54**, 65 (2004)
34. I. Bakhouche, T. Aliat, T. Boubellouta, L. Gali, A. Send, Y. Bellik, S. Afr. J. Bot. **139**, 42 (2021)
35. N. Djermane, L. Gali, R. Arhab, N. Gherraf, C. Bensouici, R. Erenler, M. Gok, A. Abdessamed, Biocatal. Agric. Biotechnol. **29**, 101834 (2020)
36. A. Szydłowska-Czerniak, C. Dianoczki, K. Recseg, G. Karlovits, E. Szłyk, Talanta **76**, 899 (2008)
37. R. Apak, K. Güclü, M. Özyürek, S.E. Celik, Microchim. Acta **160**, 413 (2008)
38. M. Rahmouni, H. Laouer, S. Dahamna, L. Gali, C. Bensouici, G. Flamini, S. Akkal, Biocatal. Agric. Biotechnol. **34**, 102017 (2021)
39. S. Lordan, T.J. Smyth, A. Soler-Vila, C. Stanton, R.P. Ross, Food Chem. **141**, 2170 (2013)
40. S. da Silva, A. Sato, C.L.S. Lage, R.A.S. SanGil, D.A. Azevedo, M.A. Esquibel, J. Braz. Chem. Soc. **16**, 1387 (2005)
41. J.A. Povh, E.O. Ono, Acta Sci. Biol. Sci. **28**, 189 (2006)
42. V.R. Affonso, H.R. Bizzo, C.L.S. Lage, A. Sato, J. Agric. Food Chem. **57**, 6392 (2009)
43. I. Grzegorczyk-Karolak, Ł. Ku, H. Wysoki (2016)
44. M. Talukdar, D.K. Swain, P.B.S. Bhadoria, Ann. Agric. Sci. **67**, 15 (2022)
45. T. Bekircan, A. Yaşar, S. Yıldırım, M. Sökmen, A. Sökmen, 3Biotech. **8**, 1 (2018)
46. J. Choi, H.J. Kang, S.Z. Kim, T.O. Kwon, S.-I. Jeong, S. Il Jang, Arch. Pharm. Res. **36**, 912 (2013)
47. R.R. Shah, K.V. Subbaiah, A.R. Mehta, Can. J. Bot. **54**, 1240 (1976)
48. F. Mirheidari, M. Hatami, M. Ghorbanpour, S. Afr, J. Bot. **145**, 323 (2021)
49. A.O. Aremu, W.A. Stirk, N.A. Masondo, L. Plačková, O. Novák, A. Pěnčík, M. Zatloukal, J. Nisler, L. Spíchal, K. Doležal, J.F. Finnie, J. Van Staden, Plant Sci. **238**, 81 (2015)
50. G.-T. Jeong, J.-C. Woo, D.-H. Park, Biotechnol. Bioprocess Eng. **12**, 86 (2007)
51. E. Karalija, A. Paric, Biol. Nyssana **2**, 29 (2011)
52. J.N.C. Vasconcelos, A.L. Brito, A.L. Pinheiro, D.I.J.G.C. Pinto, J.R.G.S. Almeida, T.L. Soares, J.R.F. de Santana, Biocatal. Agric. Biotechnol. **22**, 101408 (2019)
53. J. Deikman, P.E. Hammer, Plant Physiol. **108**, 47 (1995)
54. Y.P. Li, C.X. Yu, J. Qiao, Y.M. Zang, Y. Xiang, G.X. Ren, L. Wang, X.Y. Zhang, C.S. Liu, Rev. Bras. Farmacogn. **26**, 490 (2016)
55. Q. Liu, B. Xi, Y. Sun, W. Xu, J. Northwest Univ. Sci. Ed. **47**, 112 (2019)
56. Z. Zhang, Y. Zhang, S. Zhang, L. Wang, X. Liang, X. Wang, H. Wu, H. Zou, C. Zhang, M. Wang, J. Plant Growth Regul. **41**, 2232 (2022)
57. M. Hodaei, M. Rahimmalek, A. Arzani, M. Talebi, Ind. Crops Prod. **120**, 295 (2018)
58. Y. He, X. Zhong, X. Jiang, H. Cong, H. Sun, F. Qiao, Plant Physiol. Biochem. **156**, 461 (2020)

59. I. Weremczuk-Jeżyna, Ł Kuźma, A.K. Kiss, I. Grzegorczyk-Karolak, Acta Physiol. Plant. **40**, 1 (2018)
60. H.S. Taha, R.A. Abd El-Rahman, M. Fathalla, M. Abd-El-Kareem, U.E. Aly, Aust. J. Basic Appl. Sci. **2**, 1148 (2008)
61. F. Brahmi, F. Dahmoune, N. Kadri, M. Chibane, S. Dairi, H. Remini, S. Oukmanou-Bensidhoum, L. Mouni, K. Madani, J. Complement. Integr. Med. (2017). https://doi.org/10.1515/jcim-2016-0064
62. A. Benabdallah, C. Rahmoune, M. Boumendjel, O. Aissi, C. Messaoud, Asian Pac. J. Trop. Biomed. **6**, 760 (2016)
63. J. Bouayed, K. Piri, H. Rammal, A. Dicko, F. Desor, C. Younos, R. Soulimani, Food Chem. **104**, 364 (2007)
64. V. Katalinic, M. Milos, T. Kulisic, M. Jukic, Food Chem. **94**, 550 (2006)
65. G. Oboh, J.B.T. Rocha, Eur. Food Res. Technol. **225**, 239 (2007)
66. D. Skroza, V. Šimat, L. Vrdoljak, N. Jolić, A. Skelin, M. Čagalj, R. Frleta, G. Mekinić, Antioxidants **11**, 1784 (2022)
67. G.K. Jayaprakasha, R.P. Singh, K.K. Sakariah, Food Chem. **73**, 285 (2001)
68. S. Maina, D.H. Ryu, G. Bakari, G. Misinzo, C.W. Nho, H.Y. Kim, Antioxidants **10**, 1952 (2021)
69. S. Vladimir-Knežević, B. Blažeković, M. Kindl, J. Vladić, A.D. Lower-Nedza, A.H. Brantner, *Acetylcholinesterase Inhibitory, Antioxidant and Phytochemical Properties of Selected Medicinal Plants of the Lamiaceae Family* (Multidisciplinary Digital Publishing Institute, Basel, 2014), pp.767–782
70. I.B.H. Yahia, Y. Zaouali, M.L. Ciavatta, A. Ligresti, R. Jaouadi, M. Boussaid, A. Cutignano, Molecules **24**, 2351 (2019)
71. J. Qian, L. Guiping, L. Xiujun, H. Xincai, L. Hongmei, Nat. Prod. Res. **23**, 127 (2009)
72. M.E. Pares, Circ. Farm. **41**, 133 (1983)
73. A.T. Mata, C. Proença, A.R. Ferreira, M.L.M. Serralheiro, J.M.F. Nogueira, M.E.M. Araújo, Food Chem. **103**, 778 (2007)
74. L.B. Roseiro, A.P. Rauter, M.L.M. Serralheiro, Nutr. Aging **1**, 99 (2012)
75. S. El Aanachi, L. Gali, S. Rammali, C. Bensouici, H. Aassila, K. Dari, J. Food Meas. Charact. **15**, 1785 (2021)
76. M.H. Alu'datt, T. Rababah, M.N. Alhamad, K. Ereifej, M. Al-Mahasneh, S. Brewer, M. Rawshdeh, J. Food Biochem. **40**, 335 (2016)
77. M.B. Bahadori, G. Zengin, S. Bahadori, L. Dinparast, N. Movahhedin, Int. J. Food Prop. **21**, 198 (2018)

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH (“Springer Nature”).
Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users (“Users”), for small-scale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use (“Terms”). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.
These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.
We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.
While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
3. falsely or misleadingly imply or suggest endorsement, approval , sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
4. use bots or other automated methods to access the content or redirect messages
5. override any security feature or exclusionary protocol; or
6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.
These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.
To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.
Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.
If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com