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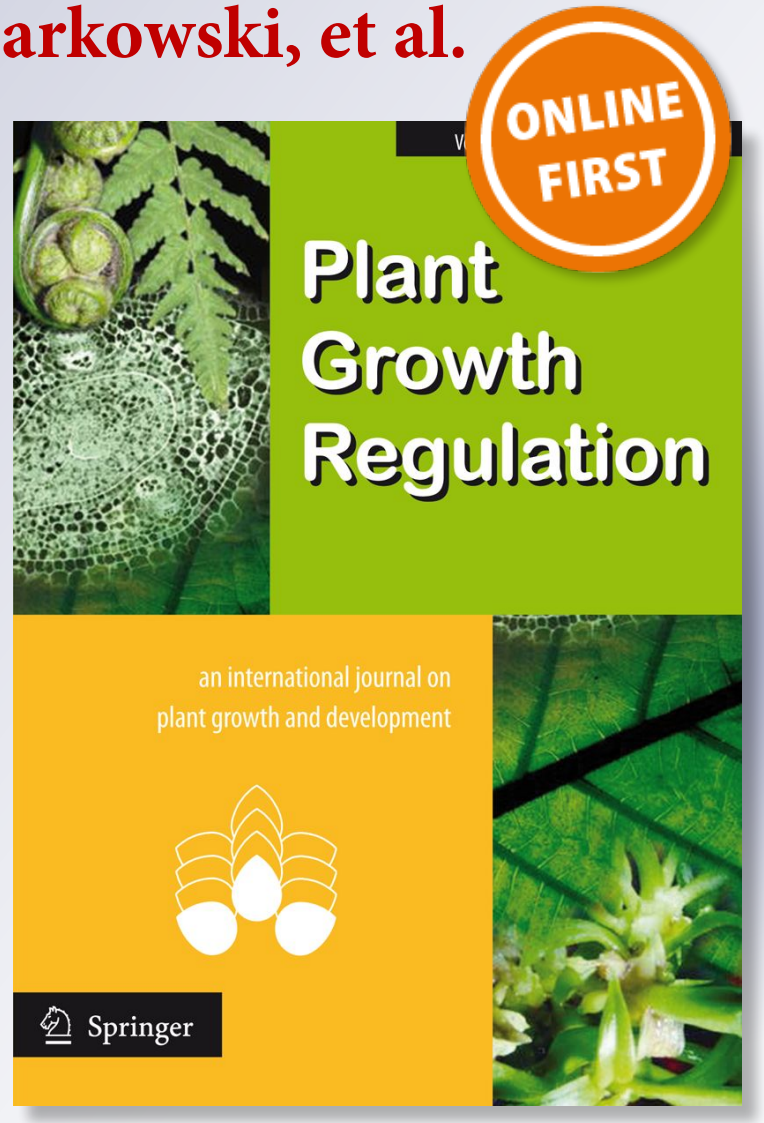

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ORIGINAL PAPER

Regulation of growth, nutritive, phytochemical and antioxidant potential of cultivated *Drimiopsis maculata* in response to biostimulant (vermicompost leachate, VCL) application

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Abstract

The effect of vermicompost leachate (VCL, low-cost biostimulant) on the growth, elemental (macro and micro-nutrients) and phytochemical content as well as the antioxidant potential of *Drimiopsis maculata* was evaluated. Three dilutions (1:5; 1:10 and 1:20) of VCL were tested and the cultivation lasted for 3 months. In addition to the recorded growth parameters, dried and ground plant materials (leaves and bulbs) were evaluated for nutrients, phenolic acids and antioxidant capacity. Vermicompost leachate application enhanced the growth of *D. maculata*, particularly, the leaves (VCL 1:10) and bulbs (VCL 1:20) which were significantly bigger than the controls. Apart from the concentration of phosphorus which was significantly lower in the leaves of VCL (1:20)-treated plants, the quantity of all four macro-nutrients analysed were similar with and without VCL. Similar observations were also demonstrated in the majority of quantified micro-nutrients in *D. maculata*. Relative to the control, VCL-treated plants had higher concentrations of the 10 phenolic acids quantified in the leaves. However, the majority of the quantified phenolic acids were not significantly enhanced in bulbs. Antioxidant activity of *D. maculata* extracts was generally higher in leaves than in the bulbs. The leaf extract from VCL (1:10 and 1:20)-treated plants exhibited lower oxygen radical absorbance capacity (ORAC) when compared to the control. However, bulbs from VCL (1:5) treatment had significantly higher ORAC than the control. From a conservational perspective, the current findings provided insight on viable approaches useful for mitigating challenges associated with over-harvesting of highly utilized but slow-growing plant species.

Keywords Asparagaceae · Conservation · Geophytes · Phenolic acids · Plant nutrients · Medicinal plants

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Abbreviations

AAPH	2,2-Azobis(2-methylpropionamidine) dihydrochloride
CRD	Completely randomised design
DW	Dry weight
ICP-MS	Inductively coupled plasma mass spectrometry
ORAC	Oxygen radical absorbance capacity
ORS	Octapole reaction system
TE	Trolox equivalents
UHPLC	Ultra-high performance liquid chromatography
VCL	Vermicompost leachate

Introduction

Globally, there is a need for effective utilization of the limited arable land to enhance plant productivity. Although the application of inorganic fertilizers and associated synthetic chemicals have been extensively utilized to increase productivity of plants, their resultant detrimental effects on the environment and residual negative effects on human health are a major concern (Lyson 2002; Tilman et al. 2002). As a result, there is an increasing call for a shift in paradigm which leans toward 'green-based' agricultural practices including the use of biostimulants (Calvo et al. 2014; Lyson 2002; Sharma et al. 2014). Biostimulants are generally sourced from diverse (natural) sources and hold tremendous economic potential with an estimated $2 billion worth of sales in the global market by 2018 (Brown and Saa 2015; Calvo et al. 2014). The application of biostimulants offers a potential novel approach to enhanced growth and development as well as mitigate biotic and abiotic stresses during the cultivation of plants with diverse attributes such as nutritional, ornamental and medicinal values (Craigie 2011; Halpern et al. 2015; Sharma et al. 2014; Yakhin et al. 2017).

Interest in the cultivation of medicinal plants have gained considerable attention as a result of the need to meet the increasing demand from both local and international markets (Affolter and Pengelly 2007; Canter et al. 2005; Lubbe and Verpoorte 2011; Moyo et al. 2015). Particularly, the intense harvesting of perennial herbs and geophytes for commercial purposes has resulted in significant depletion in the wild population of many species in sub-Saharan Africa (Dold and Cocks 2002; Moyo et al. 2015; Williams et al. 2007). As an alternative to harvesting medicinal plants from the wild population, their cultivation has the potential to overcome the challenges which entail quality control and ensure sustainability as well as availability (Canter et al. 2005; Lubbe and Verpoorte 2011; Wiersum et al. 2006). In addition, it will be valuable to devise novel approaches that have the potential to help reduce the long regeneration cycle of bulbs which are often utilized in traditional medicine (Moyo et al. 2015). Recently, researchers have demonstrated the potential of using biostimulants such as seaweed extracts, vermicompost and associated products for growth and cultivation of plants with medicinal and nutritive value (Aremu et al. 2014, 2015a, 2016; Masondo et al. 2016; Pant et al. 2012; Wang et al. 2014). Apart from enhancing the morphological appearance of cultivated plants, the phytochemical integrity of the medicinal plant need to be demonstrated in order to guarantee their acceptability by local and international consumers (Lubbe and Verpoorte 2011). Currently, there is paucity of knowledge on the phytochemical integrity of cultivated medicinal plants subjected to different biostimulants.

Drimiopsis maculata Lindl. & Paxton (syn *Ledebouria petiolata* J.C.Manning & Goldblatt; family: Asparagaceae, formerly: Hyacinthaceae) is a geophyte which is distributed from Tanzania to South Africa (Manning et al. 2004). In South Africa, the species is widespread in the eastern part of the country particularly, in provinces such as Mpumalanga, Gauteng, KwaZulu-Natal and Eastern Cape. Besides the ornamental potential of *D. maculata* (Reinten et al. 2011), it is well-known for its medicinal value among communities in South Africa. For instance, infusions prepared from the pound bulbs are applied as an enema for stomach-related ailments in children (Hulme 1954). Bulb infusions are also administered as purge for newly born suffering from '*ipleyti*', related to a marasmic condition among the Zulus (Hutchings et al. 1996). In addition, traditional healers administer the water extracts from the bulbs as enema to children with stomach ailments. Given that such ailments are often accompanied by fever, the ethno-medicinal rationale for *D. maculata* may be related to its anti-inflammatory potential. As a result, du Toit et al. (2005) isolated five (5) homoisoflavanones and structurally related compounds from *D. maculata* which were screened for anti-inflammatory activity. While compound six exhibited high anti-inflammatory activity, compounds 2, 5, 20 and 21 were moderately active in term of inhibition in microsomal cells (%). The diversity of phytochemicals in the bulb of *D. maculata* has been well-demonstrated including norlignans, scillascillin-type homoisoflavanone and xanthones (Koorbanally et al. 2001, 2006; Mulholland et al. 2004).

Given the rich chemical pool and susceptibility of *D. maculata* to over-harvesting resulting from the use of the bulbs in traditional medicine, the current study investigated how the application of a low-cost and environment-friendly biostimulant (vermicompost leachate, VCL) influences the growth, nutrient (macro and micro) composition and phytochemical content. Furthermore, the antioxidant activity of the VCL-treated plants was evaluated to provide an indication of their biological efficacy.

Materials and methods

Source of plant

Approximately 60 bulbs of *D. maculata* were collected from Silverglen Nature Reserve (Chatsworth, KwaZulu-Natal, South Africa). The identity of the plant was confirmed by Mr Brian Abrahams (Nursery Manager, Silverglen Nature Reserve) and a voucher specimen (Voucher number MUT/KKN 25) of the plant including field data was prepared and deposited at the Departmental Herbarium of Mangosuthu University of Technology, Durban, South Africa.

Preparation and analysis of vermicompost leachate

Each worm composter consisted of a 3 tier rectangular bin (145 l) made of black polyethylene plastic (Global Worming CC). The middle bin housed the bedding the worms and the feed, placed in layers as follows; first layer consisted of moistened (by soaking overnight) shredded paper forming a worm blanket to insulate the worms and prevent upward migration. The second layer consisted of dried cow dung. Then, 500 red wriggler worms (*Eisenia foetida*) measuring approximately 2.5 cm each in length were added to the composter. The worms were fed weekly with 1 kg of lettuce (*Lactuca sativa*, crisphead variety). The bottom bin served as a collection compartment for the worm leachate. The bins were kept away from direct sunlight. Approximately 1 l of water (kept in open air to remove chlorine) was added to each composter monthly in order to keep the bedding moist. Worms' leachate (VCL) was collected after four months by opening a tap at the bottom of the collection bin. The VCL was stored in a polyethylene 25 l drum in a cold room at 6 °C until application.

The pH and electrical conductivity of the VCL was measured using pH-200 pH meter and COM-80 EC meter from HM, respectively. Primary nutrients in the leachate were determined using a spectrophotometer (Cary 50 UV–Visible Spectrophotometer, Varian, Australia) following a modified method outlined by Kulkarni et al. (2014). The pH and electrical conductivity of the liquid was 8.0 and 2.5 dS/m, respectively. The dark brown leachate contained 654 mg/l of potassium (K^+), 220 mg/l of nitrate (NO_3^-) and 186 mg/l of phosphate (PO_4^{3-}).

Preparation, establishment of plants and experimental design

Healthy bulbs were planted in plastic pots (diameter of 0.30 m at the bottom and 0.47 m at the top and depth of 0.115 m) and watered daily for 2 weeks. These bulbs were left to sprout on the roof-top garden of Mangosuthu University of Technology, Durban, South Africa (S 2958.142 E 3054.768) under natural field conditions with an average temperature of 24 °C and humidity of 79% (http://www.timeanddate.com). A 40 m^2 area was prepared consisting of ten beds. Each bed had a length of 1.8 m and width of 0.8 m and accommodated ten plants. The intra-row spacing and inter-row spacing was 45 and 40 cm, respectively which was sufficient to accommodate the growth habit of the plants. Plantlet were transplanted to each bed containing potting mix (Grovida 30 dm^3) mulched into the soil. The pH of the soil ranged from 7.6 to 8.0.

Plants were arranged in a completely randomised design (CRD) consisting of ten plants (in triplicates) per treatment. Four treatments namely: control (deionised water) and 1:5, 1:10, 1:20 dilutions (i.e. 1 ml VCL + 5 ml water; 1 ml VCL + 10 ml water; 1 ml VCL + 20 ml water) of VCL obtained from red wriggler worms (*Eisenia foetida*) were applied once a week for 4 weeks to the base of each seedling.

Fifty millilitre of the treatments were applied (via soil drenching) once a week for a month (during the first month). At the end of the 3 months, morphological growth parameters (for e.g. numbers of leaves, roots and bulbs). Whole plants were thoroughly washed with distilled water to remove soil and weighed to obtain fresh weights. Dry weights were obtained after air drying for 2 weeks at room temperature.

Inductively coupled plasma mass spectrometry (ICP-MS)-based macro and micro-nutrients analysis

In triplicates, the ground plant material (about 15–25 mg) from the leaves and bulbs of *D. maculata* were weighed into polytetrafluoroethylene vessels. Thereafter, 2 ml of HNO_3 (67%, analpure) and 1 ml of H_2O_2 (30%, analytical grade) (Analytika Ltd., Prague, Czech Republic) were added and digested in diffused microwave system (MLS 1200 Mega; Milestone S.r.L., Sorisole, Italy).

The resultant solutions were diluted to 15 ml in test tubes with deionised water and analysed by ICP-MS (Agilent 7700x; Agilent Technologies, Tokyo, Japan) based on quadrupole mass analyser and octapole reaction system (ORS 3). Collision cell in He-mode was used for elimination of possible polyatomic interferences and instrument was set-up by using Tuning solution (Agilent Technologies, Santa Clara, USA). Isotopes ^{23}Na and ^{24}Mg were measured in a gas mode while isotopes ^{31}P, ^{39}K, ^{44}Ca, ^{55}Mn, ^{56}Fe, ^{63}Cu and ^{66}Zn were measured in He-mode. The internal standards included ^{6}Li, ^{45}Sc and ^{74}Ge. The calibration solutions were prepared by the appropriate dilution of the single element certified reference materials with 1.000 ± 0.002 g/l for each element (Analytika Ltd., Prague, Czech Republic) with deionised water (18.2 MΩ cm, Direct-Q; Millipore, Molsheim, France).

Certified reference materials of strawberry leaves and green algae (METRANAL® 3 and METRANAL® 8, Analytika Ltd., Prague, Czech Republic) were used for controlling the decomposition process and for method validation. Measurement accuracy was verified by using certified reference material of water TM-15.2 (National Water Research Institute, Ontario, Canada).

Ultra-high performance liquid chromatography (UHPLC)-based phenolic acid quantification

Based on the methods described by Gruz et al. (2008), the phenolic acids in the leaves and bulbs of *D. maculata* were quantified using UHPLC (Waters, Milford, MA, USA) linked to a Micromass Quattro micro™ API benchtop triple quadrupole mass spectrometer (Waters MS Technologies, Manchester, UK). Briefly, in triplicates, the ground plant materials were homogenized with 80% methanol (40 mg/ml) in a 1.5 ml Eppendorf tube, using an oscillation ball mill (MM 301, Retsch, Haan, Germany) at a frequency of 25 Hz for 3 min. Deuterium-labelled internal standards were added to the extraction solvent prior to plant material homogenization. The extracts were centrifuged for 10 min at 26,000*g* and the supernatant was filtered through 0.45 µm nylon micro-filters (Alltech, Breda, Netherlands).

Oxygen radical absorbance capacity (ORAC) assay

Following 80% methanol extraction of ground plant materials from the leaves and bulbs of *D. maculata*, ORAC of the resultant extracts was measured using the protocol developed by Ou et al. (2001). In triplicates, fluorescein (100 µl, 500 mM) and plant extracts (25 µl) were added into each working well in a 96-well microplate and shaken. The reaction was initiated by the addition of 2,2-Azobis(2-methylpropionamidine) dihydrochloride (AAPH, 25 µl, 250 mM) pre-incubated at 37 °C. The fluorescence (Ex. 485 nm, Em. 510 nm) was read every 3 min over 90 min in a microplate reader Infinite M200 Pro (Tecan, Switzerland) incubated at 40 °C. The net area under the curve was used to calculate antioxidant capacity in trolox equivalents (µmol TE/g).

Data analysis

Growth response, elemental and phytochemical content as well as antioxidant activity data were subjected to analysis of variance (ANOVA) using SPSS software version 22.0. For each of the evaluated parameters, the mean values were further separated using Duncan's multiple range test to check for statistical significance ($P \leq 0.05$).

Results

Growth data

Application of different dilutions of VCL improved the morphological traits of *D. maculata* (Fig. 1). For instance, VCL-treated plants had more leaves which also translated to higher fresh and dry biomass (Fig. 1a–c). Similarly, VCL treatment stimulated the production of higher numbers of shoots in *D. maculata* (Fig. 1d). However, the bulb biomass was only significantly higher in VCL (1:20) treatments (Fig. 1e, f).

Macro- and micro-nutrient composition

The concentrations of the four macro-nutrients analysed in *D. maculata* were generally higher in the leaves than in the bulbs (Fig. 2). The degree of abundance were in order of K > Ca > P > Mg in both organs of *D. maculata*. In most cases, no significant increase in the concentrations of macro-nutrients was observed in VCL-treated plants (Fig. 2a–d, f–h). However, the P content was remarkably lower in leaves of VCL (1:20) treatment than in the control (Fig. 2e).

As depicted in Fig. 3a–j, the leaves accumulated higher concentrations of micro-nutrients than the bulbs regardless of the treatment regime. While Na was the most abundant (730–3366 µg/g DW) micro-nutrient, Mn occurred in the least concentration (10–20 µg/g DW). An increase in Cu content was observed in leaves of VCL (1:5)-treated *D. maculata* (Fig. 3a). However, the same treatment (VCL 1:5) resulted in lower Fe content in leaves (Fig. 3c). In the bulbs, 1:10 and 1:20 (dilutions) VCL treatments increased the Mn and Zn contents, respectively (Fig. 3f, j).

Phenolic acid content

The ten phenolic acids quantified in the extracts from the two organs of *D. maculata* were classified as either hydroxybenzoic (Fig. 4) or hydroxycinnamic (Fig. 5) based on their chemical structures. In most cases, both classes of phenolic acid (with the exception of ferulic acid) accumulated in higher concentrations in the leaves than in the bulbs. In terms of quantity, *p*-hydroxybenzoic (11–19 µg/g) and vanillic (13–22 µg/g) acids were the major hydroxybenzoic derivatives while *p*-coumaric (28–41 µg/g) and ferulic (5–8 µg/g) acids were the most abundant hydroxycinnamic derivative. The remaining phenolic acids were generally in low concentrations (< 5 µg/g) in both the leaves and bulbs of *D. maculata*.

Among the six hydroxybenzoic quantified in the leaf extracts of *D. maculata*, VCL application significantly enhanced the phenolic acid content relative to the control

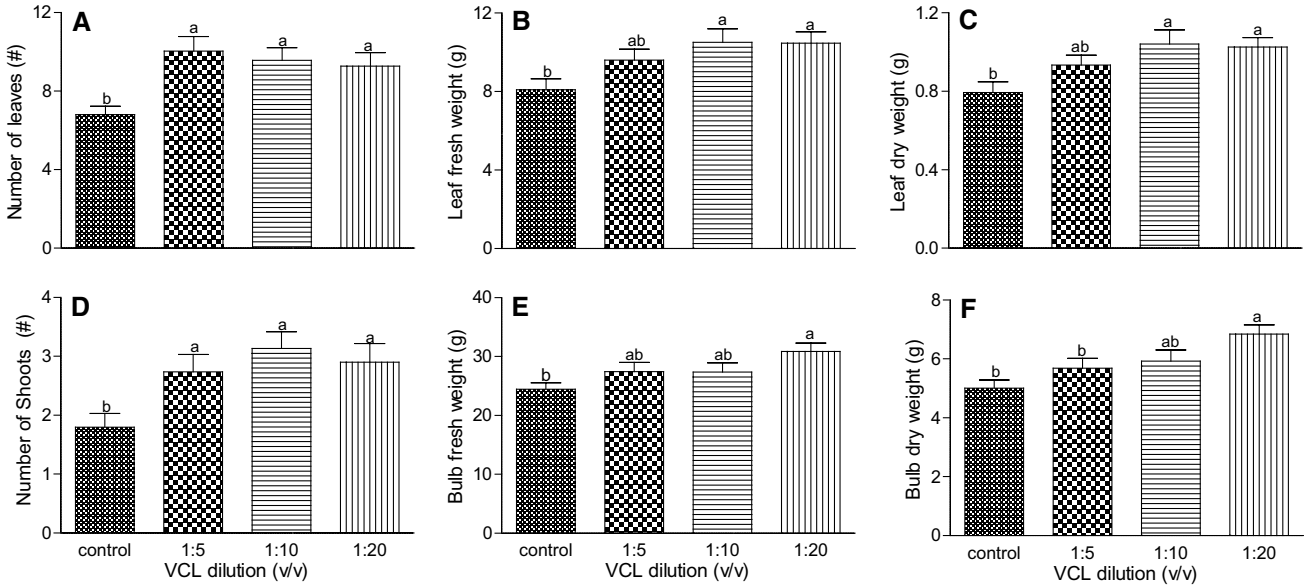

Fig. 1 Effect of different dilutions of vermicompost leachate (VCL) on morphological traits of *D. maculata*. **a** Number of leaves; **b** leaf fresh weight; **c** leaf dry weight; **d** number of shoots; **e** bulb fresh weight; **f** bulb dry weight. Data are presented as mean ± standard error ($n = 30$). In each graph, bars with different letter(s) are significantly different based on Duncan's multiple range test ($P \leq 0.05$)

(Fig. 4a, c, e, g, i, k). Similarly, gallic acid content in the bulb extracts was higher in VCL (1:5)-treated plants (Fig. 4b). Conversely, VCL application reduced the concentration of *p*-hydroxybenzoic, protocatechuic, salicylic, syringic and vanillic acids in the bulb extracts (Fig. 4d, f, h, j, i).

In the leaf extracts, VCL application resulted into higher concentration of the four hydroxycinnamic acids relative to the control (Fig. 5a, c, e, g). Although VCL (1:20) enhanced the caffeic acid in bulbs (Fig. 5b), no significant increase was observed in the bulb extracts for the other three hydroxycinnamic acids (Fig. 5d, f, h).

Antioxidant activity

In terms of the ORAC, the extracts from the leaves were more potent when compared to the bulbs (Table 1). Application of VCL had no significant stimulatory effect on the ORAC of the extracts from the leaves of *D. maculata*. In fact, lower dilutions (1:10 and 1:20) of VCL reduced the ORAC of the leaf extracts. On the other hand, bulb extracts prepared from VCL (1:5) treatment had higher ORAC activity than the control.

Discussion

Effect of vermicompost leachate (VCL) application on growth response

The beneficial effect of biostimulants including VCL are often manifested over the life cycle of the plant. For instance, effects such as improved seed germination, seedling establishment, enhanced nutrient mobilization and partitioning, improved rooting of cuttings, flowering, fruit and crop yield have been demonstrated across a wide range of plants (Craigie 2011; Halpern et al. 2015; Sharma et al. 2014). This is not surprising as these products are sourced from diverse organisms and generally contain a wide range of growth-stimulatory metabolites (Aremu et al. 2015b; Brown and Saa 2015; Yakhin et al. 2017). In addition to compounds such as humic acid as well as micro and macro-nutrients, VCL contains cocktails of conventional plant hormones which are possibly the active ingredients and responsible for growth stimulatory effects (Aremu et al. 2015b; Zhang et al. 2014).

In the current study, VCL-treated *D. maculata* generally had bigger leaves and bulbs when compared to the control (Fig. 1). Given that biomass accumulation is a function of photosynthesis, it is possible to infer that the application of VCL influenced this important process as demonstrated in many species (Aremu et al. 2014; Arthur et al. 2012; Ievinsh 2011; Masondo et al. 2016). For instance, the reduction of photosynthetic pigment content was significantly attenuated by supplementing phosphorus-deficient *Tulbaghia ludwigiana* with VCL (Aremu et al. 2014). Given the ability of VCL to enhance morphological appearance of different plant organs especially the underground part (e.g. bulbs), it affords a viable approach useful for low-resource farmers to enhance the cultivation of bulbous plants which are often in high demand by medicinal plant vendors and users.

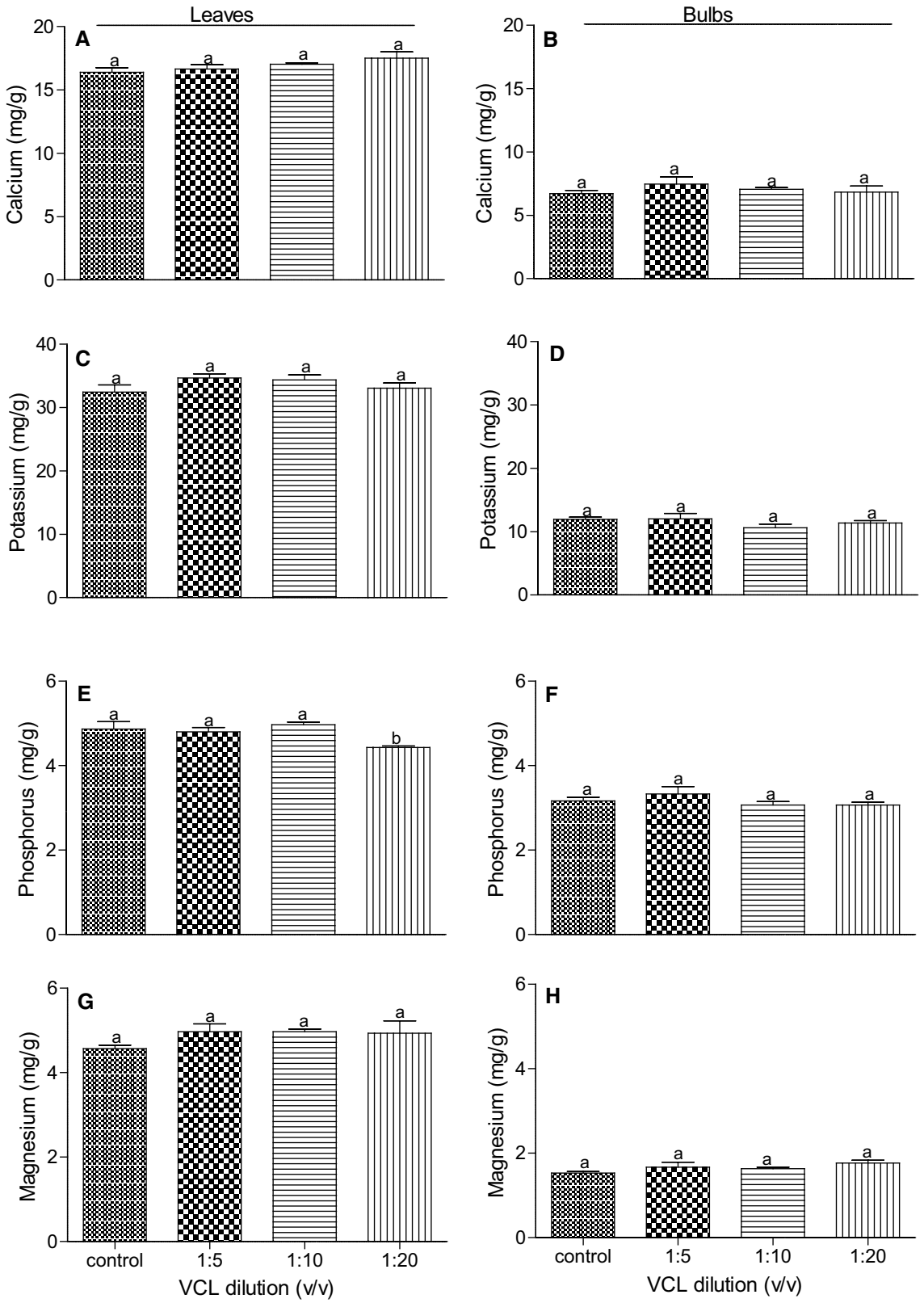

Fig. 2 Effect of different dilutions of vermicompost leachate (VCL) on the concentration of macro-nutrients in leaves and bulbs of *D. maculata*. **a, b** Calcium; **c, d** potassium; **e, f** phosphorus; **g, h** magnesium. Data are mean ± standard error ($n = 3$). In each graph, bars with different letter(s) are significantly different based on Duncan's multiple range test ($P \leq 0.05$)

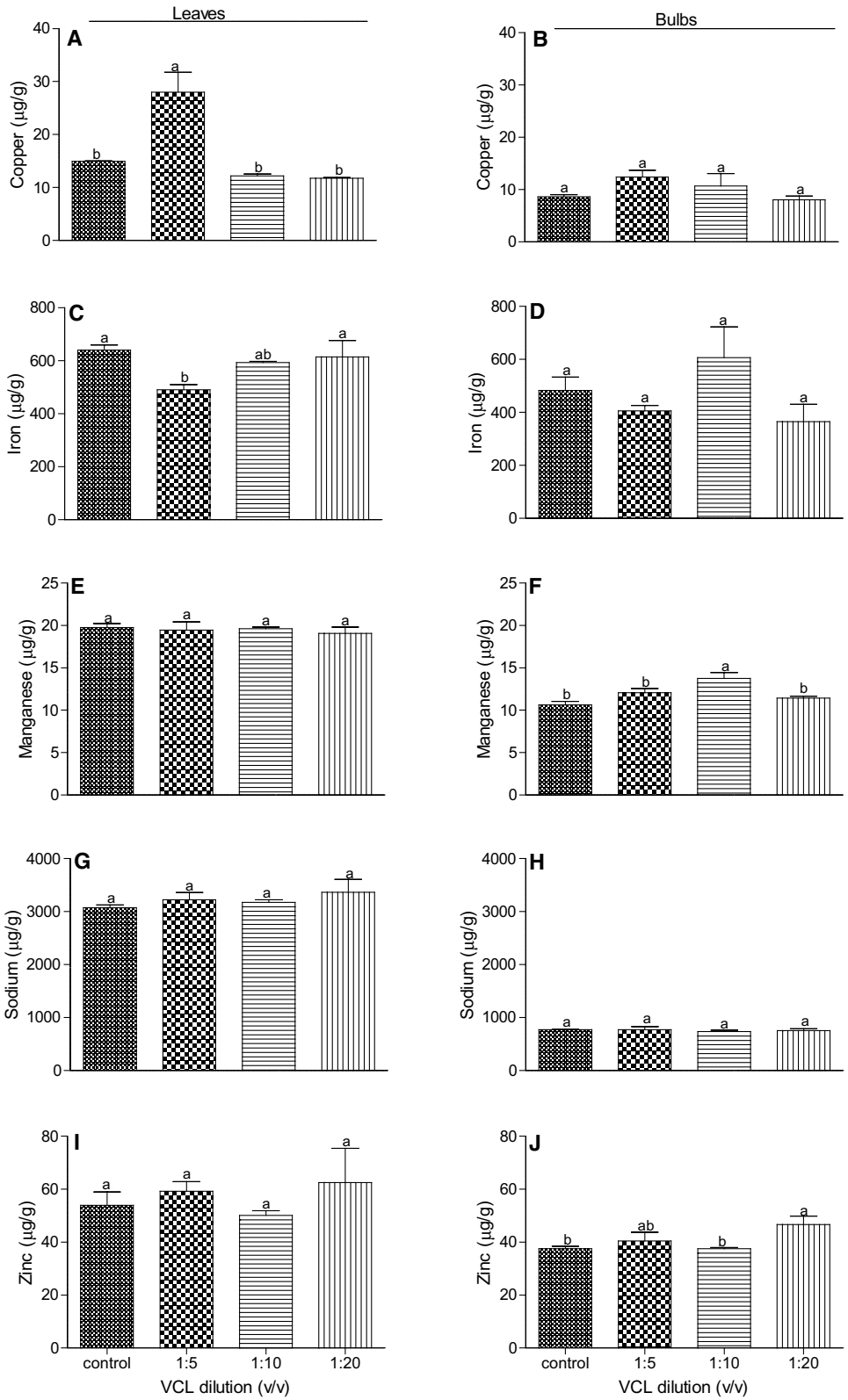

Fig. 3 Effect of different dilutions of vermicompost leachate (VCL) on the concentration of micro-nutrients in leaves and bulbs of *D. maculata*. **a, b** Copper; **c, d** iron; **e, f** manganese; **g, h** sodium; **i, j** zinc. Data are mean ± standard error ($n = 3$). In each graph, bars with different letter(s) are significantly different based on Duncan's multiple range test ($P \leq 0.05$)

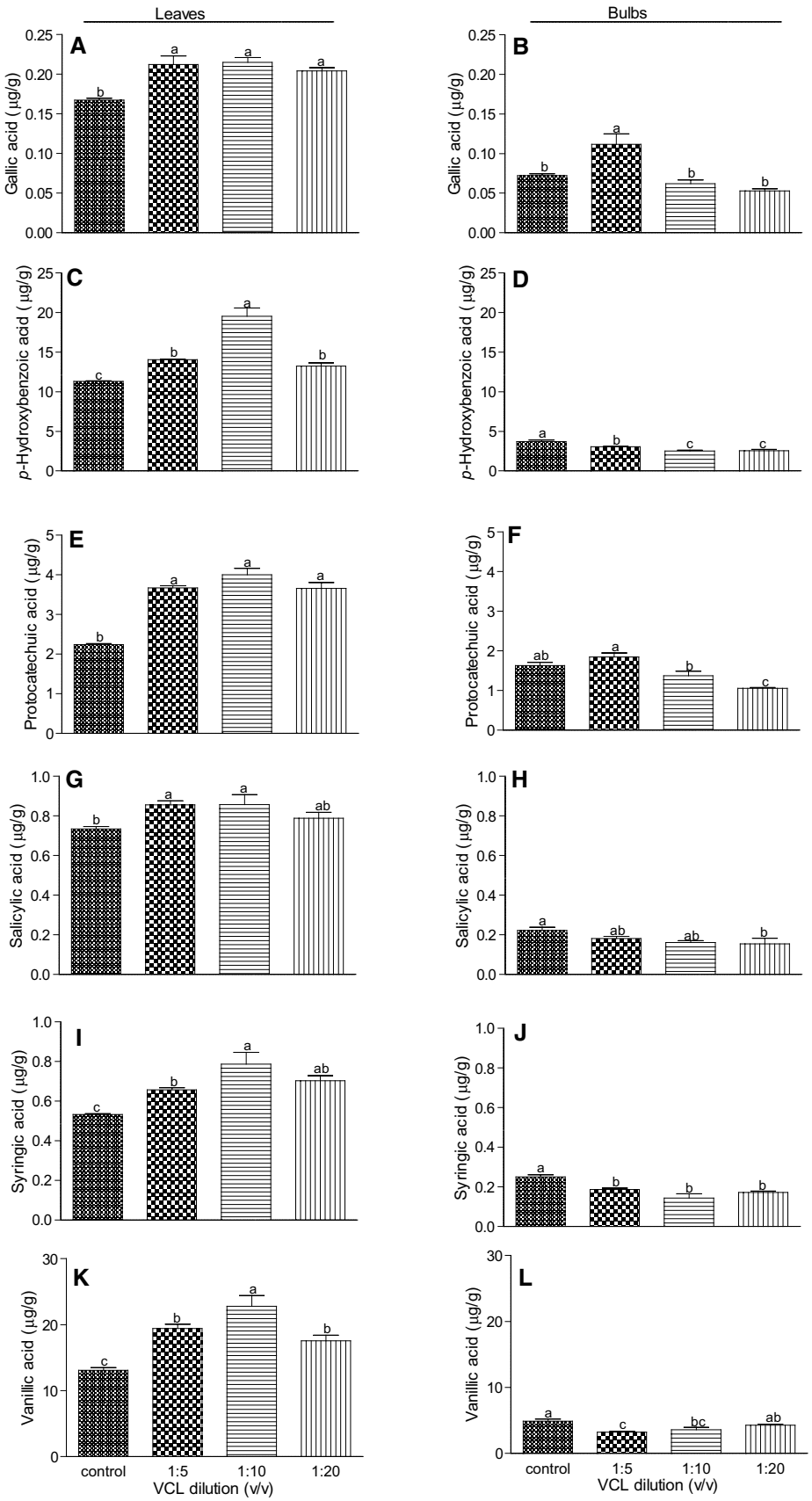

Fig. 4 Effect of different dilutions of vermicompost leachate (VCL) on the concentration of hydroxybenzoic acid derivatives in leaves and bulbs of *D. maculata*. **a, b** Gallic acid; **c, d** *p*-hydroxybenzoic acid; **e, f** protocatechuic acid; **g, h** salicylic acid; **i, j** syringic acid; **k, l** vanillic acid. Data are mean ± standard error ($n = 3$). In each graph, bars with different letter(s) are significantly different based on Duncan's multiple range test ($P \leq 0.05$)

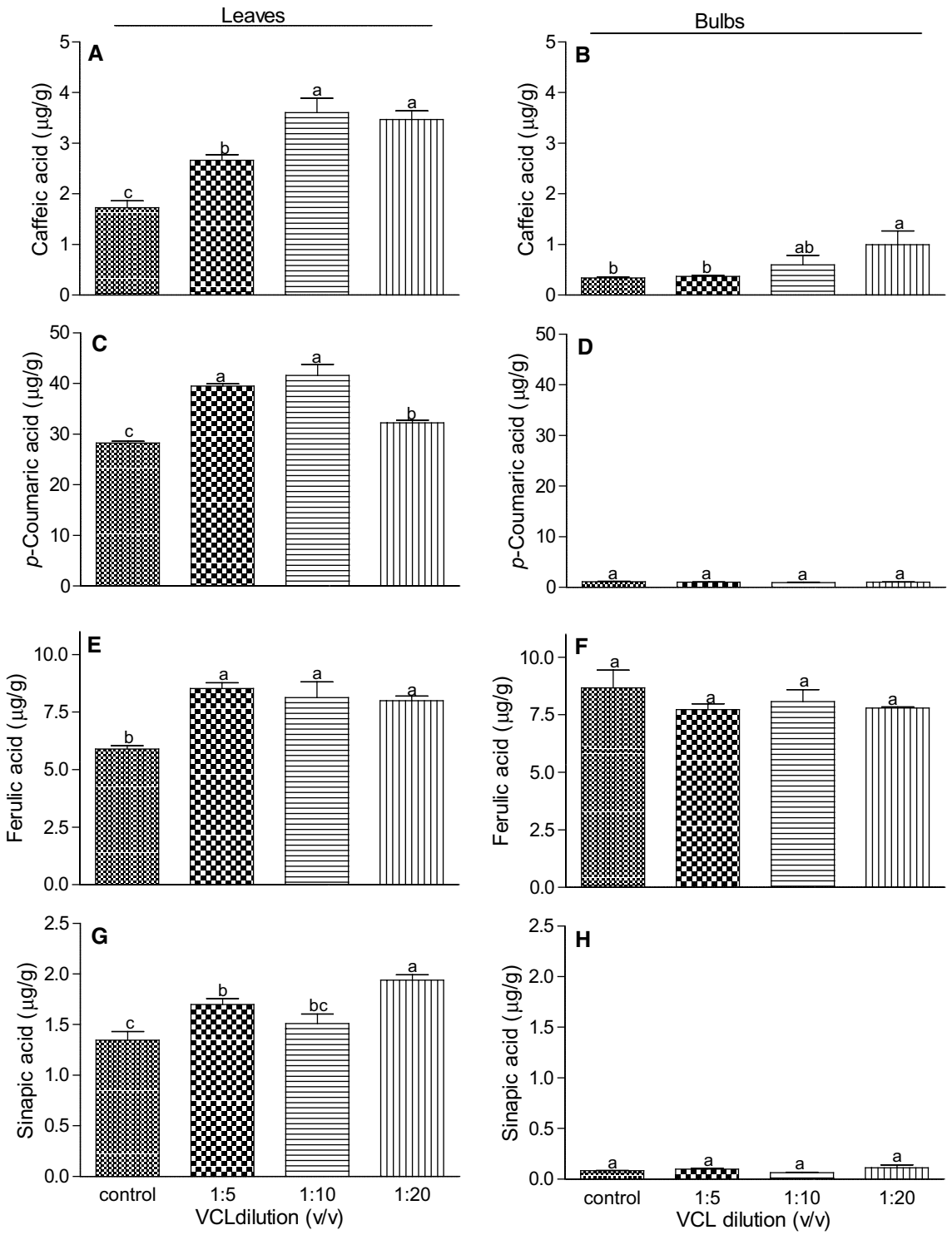

Fig. 5 Effect of different dilutions of vermicompost leachate (VCL) on the concentration of hydroxycinnamic acid derivatives in leaves and bulbs of *D. maculata*. **a, b** Caffeic acid; **c, d** *p*-coumaric acid; **e, f** ferulic acid; **g, h** sinapic acid. Data are mean ± standard error ($n = 3$). In each graph, bars with different letter(s) are significantly different based on Duncan's multiple range test ($P \leq 0.05$)

Table 1 Effect of different dilutions of vermicompost leachate (VCL) on oxygen radical absorbance capacity (ORAC) of extracts from different organs of *D. maculata*

VCL dilutions (v/v)	ORAC (μmol TE/g)	
	Leaves	Bulbs
Control	151.0 ± 9.36^{a}	23.6 ± 1.28^{b}
1:5	134.6 ± 7.23^{ab}	32.6 ± 3.40^{a}
1:10	121.7 ± 6.88^{b}	26.1 ± 0.92^{ab}
1:20	88.0 ± 2.87^{c}	26.8 ± 1.55^{ab}

In each column, value (mean ± standard error, $n = 3$) with different letter(s) are significantly different based on Duncan's multiple range test ($P \leq 0.05$)

TE trolox equivalents

Effect of vermicompost leachate (VCL) application on the concentration of macro- and micro-nutrients

The influence of biostimulant application on plant nutrient uptake and the underlying mechanisms are associated to positive changes in soil structure or nutrient solubility, root morphology and plant physiology (Halpern et al. 2015; Martínez-Ballesta et al. 2010). As emphasized by these authors, the minerals in plants are related to the effect of agricultural practices, especially the continuous debatable issue of organic farming versus mineral fertilisation. In the current study, the concentrations of macro-nutrients in *D. maculata* were similar with and without VCL treatment. However, the macro-nutrients were generally higher in the leaves than bulbs (Fig. 2). Similar trends were evident in the concentrations of micro-nutrients with few exceptions. For instance, VCL application increased the concentration of Cu in the leaves (VCL 1:5) and Mn in bulbs (VCL 1:10) of *D. maculata*. In contrast, the leaves of VCL (1:5)-treated plants had significantly lower Fe content when compared to the control. As emphasized by Martínez-Ballesta et al. (2010), this type of variable response has been demonstrated in many plants. The effect of biostimulants on nutrient (macro and micro) content are often variable depending on critical factors such as the crop, season cycle and year. Thus, these aforementioned factors must be considered carefully prior to making conclusions and recommendations to stakeholders.

Effect of vermicompost leachate (VCL) application on the phenolic acids (hydroxybenzoic and hydroxycinnamic derivatives)

The therapeutic value of phytochemicals in *D. maculata* is well recognized (Koorbanally et al. 2001). In addition, several studies have also highlighted the diversity of chemicals present in *D. maculata* (du Toit et al. 2005; Koorbanally et al. 2001, 2006; Mulholland et al. 2004). In the current study, ten phenolic acids comprising six hydroxybenzoic and four hydroxycinnamic derivatives occurred in the leaves and bulbs of *D. maculata*. Most of these phenolic acids are recognised as a potent bioactive compounds for treating different diseases (Heleno et al. 2015). Ferulic acid was one of the major phenolic acid in *D. maculata* and its diverse biological effect such as antioxidant, anti-inflammatory, antimicrobial, anti-allergic and hepato-protective effects have been demonstrated (Kumar and Pruthi 2014).

As a result of the value associated with elevated level of phytochemicals in plants, researchers often explored different approaches especially the application of different biostimulants during cultivation (Aremu et al. 2014, 2015a, 2016; Pant et al. 2012; Wang et al. 2014). Application of VCL influenced the concentrations of phenolic acid accumulated in the leaves and bulbs of *D. maculata* (Figs. 4, 5). It has been established that the type and concentration of phytochemicals in cultivated plants are often affected by different factors. For instance, the phenolic compounds in marionberry, strawberry and corn were significantly higher when grown with organic supplements compared with non-organic plants (Asami et al. 2003).

Effect of vermicompost leachate (VCL) application on antioxidant activity

Although biological screening efficacy of *D. maculata* is limited, the anti-inflammatory potential have been demonstrated (du Toit et al. 2005). Findings from the present study provided an indication of the antioxidant potential. Antioxidant activity is one of the most common biological activity exhibited by many plants and this has increased the interest in plant-derived antioxidants (Gülçin 2012). On the basis of the effect of oxidative stress in the aetiology of many diseases (Pham-Huy et al. 2008; Spector 2000), the presence of potent antioxidants in medicinal plants is desired. The antioxidant activity of cultivated plants are affected by different factors including type of fertilizer applied, necessitating the extensive focus in this area (Aremu et al. 2014; Masondo et al. 2016; Pant et al. 2009; Rimmer 2006; Toor et al. 2006; Wang et al. 2010). In the current study, application of VCL (1:5 dilution) significantly enhanced the antioxidant activity of bulb extract of *D. maculata*. However, similar higher antioxidant activity was absent in leaf extracts. In fact, the antioxidant activity demonstrated was significantly lower in leaves obtained from VCL (1:20)-treated plants. This finding indicates that the positive influence of biostimulants on antioxidant activity cannot be generalized as differences may occur in the different organs of the same plant and/or among different plants (plant specific). This plant specific-effect in antioxidant response following treatment with biostimulants have been documented in some studies (Aremu et al. 2014; Asami et al. 2003; Masondo et al. 2016).

Conclusions

Findings from the current study established the beneficial effects from the application of low-cost biostimulants such as VCL which enhanced the morphological appearance of *D. maculata*. In addition, VCL application influenced the quantity of macro and micro-nutrients as well as the accumulated bioactive phytochemicals. This implies that the use of VCL has the potential to ensure the desired quality and quantity of cultivated medicinal plants. In terms of the distribution of chemical metabolites and ORAC potential, the higher content and antioxidant demonstrated by the extracts from the leaves relative to the bulbs provides a motivation to support the call for plant part substitution (i.e. the use of leaves rather than the bulbs) in the usage of *D. maculata*. Overall, the present findings are valuable from a conservational perspective as useful insight for mitigating challenges associated with over-harvesting of highly utilized but slow-growing species especially geophytes.

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Author contributions LD, KKN, GDA and AOA conceived and conducted the field experiment. MJ and JG quantified the phenolic acids and ORAC assay. MJ and PT conducted the elemental analysis. LD, KKN, GDA and AOA analysed the data on growth parameters. KD was also involved in conceptualization, design and provided technical and editorial inputs. AOA wrote the manuscript with help from all the other authors.

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