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Phytotoxic phenylpropanoids isolated from *Ophryosporus charua* (Griseb.) Hieron.

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ABSTRACT

Bioguided isolation of the EtOH extract from the medicinal native plant, *Ophryosporus charua*, against *Raphanus sativus*, yielded three phenylpropanoids, charuol A [(*Z*)-4-((1*S*,2*R*)-3-acetoxy-1,2-dihydroxypropyl)phenyl) 2-methylbut-2-enoate], charuepoxide [(*Z*)-4-((2*S*,3*R*)-3-(acetoxymethyl oxiran-2-yl)phenyl) 2-methylbut-2-enoate] and charuol B [(*Z*)-4-((1*R*,2*R*)-3-acetoxy-1,2-dihydroxypropyl)phenyl) 2-methylbut-2-enoate]. Their structures and absolute configuration were established by extensive spectroscopic analyses. The effective concentrations for 50% inhibition of germination (ECg_{50}) and root (ECr_{50}) and shoot (ECs_{50}) elongations were determined for these compounds against *P. miliaceum* (monocot) and *Raphanus sativus* (dicot). Charuol A was the most active in the inhibition of germination of *P. miliaceum* (ECg_{50} = 0.97 mM), followed by charuol B and charuepoxide, although charuol B was the most effective in regulating the root growth of *P. miliaceum* seedlings, with an ECr_{50} of 1.0 mM. Charuol A inhibited the germination of *R. sativus*, while its seedling development was also affected by all three compounds with different effectiveness. Charuol A was also highly effective in the 0.09–0.30 mM range against other test species such as *Lactuca sativa*, *Eruca sativa*, *Allium ampeloprasum* and *Secale cereale*.

1. Introduction

Plants produce many different secondary metabolites that are involved in interaction with other organisms. Some interact with other plants, interfering with their germination and growth. The active principles for this phytotoxic effect can be allelochemicals and it is widely accepted that those that are highly efficient can be used as natural herbicides (Morimoto et al., 2009).

In a search for plants with high phytotoxic activity, 101 native plant species of Central Argentina (Palacios et al., 2007, 2010) were screened. Among the plants assayed, *Ophryosporus charua* (Griseb.) Hieron. (Astereceae) proved to be one of the most promising species, showing high inhibition of germination and of seedling growth, especially of dicotyledonous *Raphanus sativus* (Palacios et al., 2010). *O. charua* is a medicinal plant used as an antisyphilitic in traditional medicine (Toursarkissian, 1980). The ethanolic extract of this plant species was also assayed against insects (Del Corral et al., 2014; Diaz Napal et al., 2015), bacteria (Joray et al., 2011), fungi (Carpinella et al., 2010b) and some enzymes (Carpinella et al., 2010a; Chiari et al., 2010) with poor results. The *O. charua* extract was, however, a good inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity, an enzyme involved in the tyrosine degradation pathway in mammals as well as in the production of plastoquinone and tocopherol in plants (Chiari et al., 2015). Flavones, *ent*-labdanes, *ent*-halimanes (Favier et al., 1997), benzofurans, prenylated chromanones and prenylated acetophenone (de Lampasona et al., 1997) were isolated from this species but, as currently known, no bioactivity was reported for these compounds.

As part of a search for natural phytotoxic compounds (del Corral

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et al., 2012; Palacios et al., 2010), the ethanolic extract of *O. charua* was chosen for further investigation based on the previous screening results. Reported here are the bioguided isolation of three novel phenylpropanoids, **1**, **2** and **3**, as the phytotoxic principles of *O. charua* aerial tissues.

2. Results and discussion

The ethanol extract from leaves of *O. charua* (yield 13%) was subjected to liquid-liquid partition to obtain diethyl ether and aqueous subfractions. Comparison of its germination inhibitory activity on *R. sativus* indicated that the major activity of the original extract appeared in the diethyl ether fraction (yield 86%, percent of germination inhibition (IG%) = 93). This bioactive fraction was subjected to flash silica gel column chromatography, and the greatest activity was observed in a fraction eluted with hexane/Et_2O (80:20) (IG% = 94). Then, this fraction was further separated by circular preparative thin layer chromatography, and fractions 9, 17 and 28, eluted with hexane/Et_2O (85:15), showed highest inhibitory activity (IG% = 95–100). The active fractions were further purified by column chromatography and 3 new phenylpropanoids (**1**, **2** and **3**) (Fig. 1) were isolated.

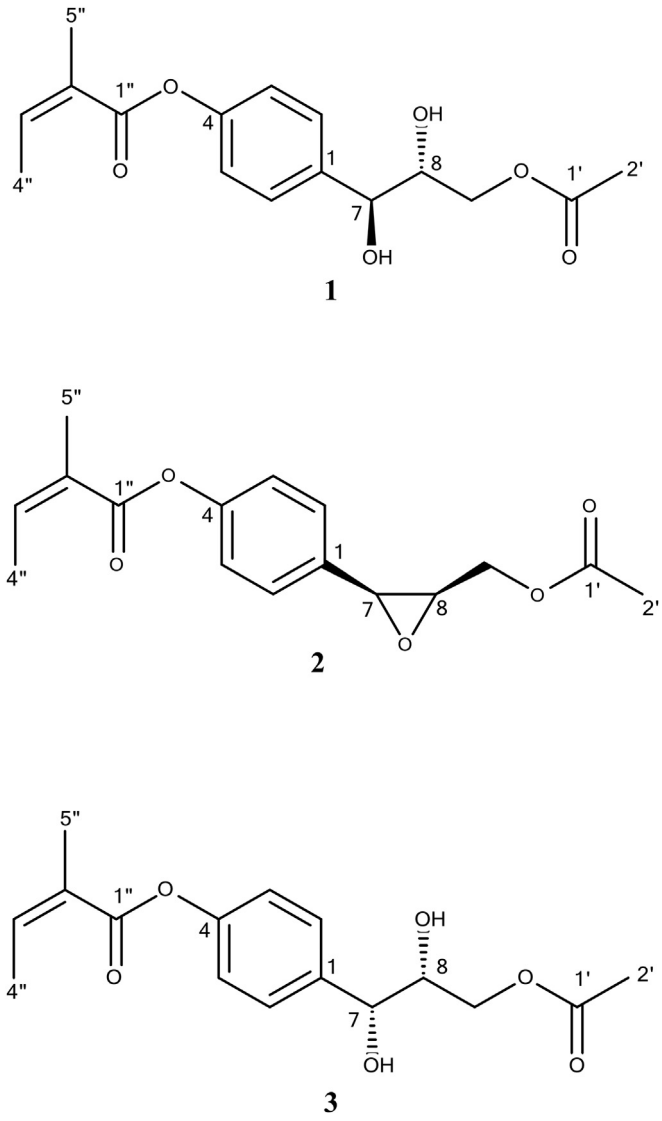

Fig. 1. Structures of compounds **1**, **2** and **3**.

Compound **1** was obtained as colorless oil. Its IR spectrum indicated the presence of hydroxyl (3475 cm^{-1}) and two carbonyl (1736.32 and 1722.13 cm^{-1}) functional groups as well as an aromatic moiety (1651.14, 1505.61 cm^{-1}). Its molecular formula, $C_{16}H_{20}O_6$, was determined from the positive-ion HR-ESI-MS (331.11546 $[M + Na]^+$, calcd 331.11521). In the ^1H NMR spectrum (Table 1), the presence of a 1,4-substituted aromatic ring was deduced from the signals at δ_H 7.41 (2H, d, J = 8.4 Hz, H-2 and H-6), 7.11 (2H, d, J = 8.4 Hz, H-3 and H-5). A *trans*-olefinic moiety was deduced from the resonances at δ_H 6.27 (1H, dd, J = 1.6, 7.2 Hz, H-3″) and was supported by corresponding signals at δ_C 140.9 (C-3″), 127.6 (C-2″) in the HSQC and HMBC spectra. This group, together with two methyl groups, δ_H 2.08 (3H, dd, J = 1.4, 8.6 Hz, H-5″) and 2.03 (3H, dd, J = 1.4, 3, H-4″), and the carboxylic resonance at δ_C 166.28 (C-1″) evidenced in the HMBC spectrum, indicated the presence of an angeloyl group esterifying the *p*-OH aromatic group. The resonances at δ_H 4.21 (1H, dd, J = 7, 11.7 Hz, H-9a), 4.13 (1H, dd, J = 3, 11.7 Hz, H-9b), 4.70 (1H, d, J = 5.6 Hz, H-7) and 4.08 (1H, m, H-8), and signals at δ_C 65.07 (C-9), 73.54 (C-8) and 74.01 (C-7) suggested presence of a propanetriol moiety that was also attached to the phenyl ring in the *para*-position to the OH-group. A carbonyl carbon at δ_C 171.66 (C-1′) was also observed in the ^{13}C NMR spectrum corresponding to an acetyl group, together with the resonance for the methyl group at δ_H 2.06 (3H, s, H-2′). The HMBC of this carbonyl with H-9, indicated that the acetyl group was attached to the C-9 hydroxyl group. In order to determine the stereochemistry of C-7 and C-8, the *erythro* configuration was first deduced from the coupling constant of H-7 (J = 5.6 Hz) in ^1H NMR spectrum (Ishikawa et al., 2002; Shu et al., 2015).

The absolute configuration of the chiral centers C-7 and C-8 in **1** was established using the modified Mosher's method, i.e., forming 7,8-di-*R*-MTPA and 7,8-di-*S*-MTPA esters of **1** (Liu et al., 2015; Seco et al., 2001). In both syntheses, just one compound was obtained, indicating that **1** was a pure diastereomer. Given the differences in chemical shifts of H-9a in the *R*- and *S*-MTPA ester of **1** ($\Delta\delta^{RS}$ = + 0.31), the *R*-configuration was attributed to C-8 (Seco et al., 2001). This was supported by the calculation of the most stable conformer for the di-MTPA substituted **1** (Fig. 2), which showed a clear shielding of H-9 due to the proximity of the phenyl moiety of the *S*-MTPA group attached to the hydroxyl group at C-8 (Fig. 2A). In the case of the *R*-MTPA derivative, however, the CF_3 group was in the proximity of the methylenic C-9 (Fig. 2B), resulting in a ^1H NMR signal at a higher chemical shift for the methylene protons. With the configuration of C-8 established as *R*-, the C-7 was deduced to be *S*-, due to the *erythro* configuration. In summary, it is proposed that **1** has a 7*S*,8*R* configuration. Compound **1** also showed a positive CD effect at 250, 261 and 268 nm. The yield of **1** was 135 mg/100 g of dried plant material as determined by HPLC of crude extract.

Compound **2** was isolated as colorless oil and its molecular formula was determined as $C_{16}H_{18}O_5$ by HR-ESI-MS. Its IR spectrum indicated the presence of two carbonyl (1739.87 and 1722.13 cm^{-1}) and aromatic functional groups (1644.04 and 1517.71 cm^{-1}). The ^1H NMR spectrum (Table 1) was identical to that of compound **1**, but clear differences were seen in the chemical shift of H-7 [δ_H 3.82 (1H, d, J = 1.8 Hz)], H-8 [δ_H 3.24 (1H, m)] and C-7 [δ_C 56.12], C-8 [δ_C 59.32]. The upfield shift of H-7 and H-8 suggested the presence of an epoxide moiety in the C-7C-8 position. The small J value (1.8 Hz) of H-7 indicated the presence of a *cis* epoxide (Zacchino, 1994), and the negative rotation angle together with a positive Cotton effect at 251, 263 and 268 nm suggested a configuration 7*S*,8*R* for the epoxy moiety. As the epoxide could have been formed during the chromatographic manipulation, its natural presence was explored by HPLC of the crude extract where it was established to be present in a yield of 45 mg/100 g of dried plant material. Thus, it is assumed

Table 1
^{1}H and ^{13}C NMR spectroscopic data and assignment of compounds **1** and **2** at 400 MHz in $DCCl_3$ (δ in ppm, *J* in Hz).

	1		**2**		**3**	
	δ_H (mult, *J*, Hz)[a]	δ_C[b]	δ_H, mult, (*J*, Hz)[a]	δ_C[b]	δ_H (mult, *J*, Hz)[a]	δ_C[b]
1	–	137.35	–	133.63	–	133.65
2	7.41 d (8.4)	127.05	7.31 d (8.8)	126.73	7.47 d (8)	129.95
3	7.11 d (8.4)	121.93	7.11 d (8.8)	122.03	7.15 d (8)	122.14
4	–	150.43	–	150.84	–	151.04
5	7.11 d (8.4)	121.93	7.11 d (8.8)	122.03	7.15 d (8)	122.14
6	7.11 d (8.4)	127.05	7.31 d (8.8)	126.73	7.47 d (8)	129.95
7	4.70 d (5)	74.01	3.82 d (1.8)	56.12	4.91 d (7)	61.86
8	4.08 d (5)	73.54	3.24 m	59.32	4.22 m	73.63
9	4.21 dd (7, 11.7)	65.07	4.46 dd (3.2, 12.2)	64.07	4.35 d (4)	65.12
	4.13 dd (3, 11.7)		4.10 dd (5.8, 12.3)		4.29 d (5.6)	
1′	–	171.66	–	170.71	–	171.05
2′	2.06 s	20.60	2.12 s	20.76	2.11 s	20.81
1″	–	166.28	–	166.10	–	165.95
2″	–	127.63	–	127.03	–	127.38
3″	6.27 dd (1.6, 7.2)	140.98	6.27 dd (1.6, 7.2)	140.95	6.29 dd (7.4)	141.13
4″	2.08 dd (1.4, 8.4)	20.85	2.07 dd (1.6, 7.3)	20.61	2.07 dd (1.4, 7.4)	20.57
5″	2.03 dd (1.4, 3)	16.01	2.04 t (1.6, 1.5)	16.01	2.04 t (1.6, 1.4)	16.01

[a] Recorded at 400 MHz.
[b] Recorded at 100 MHz.

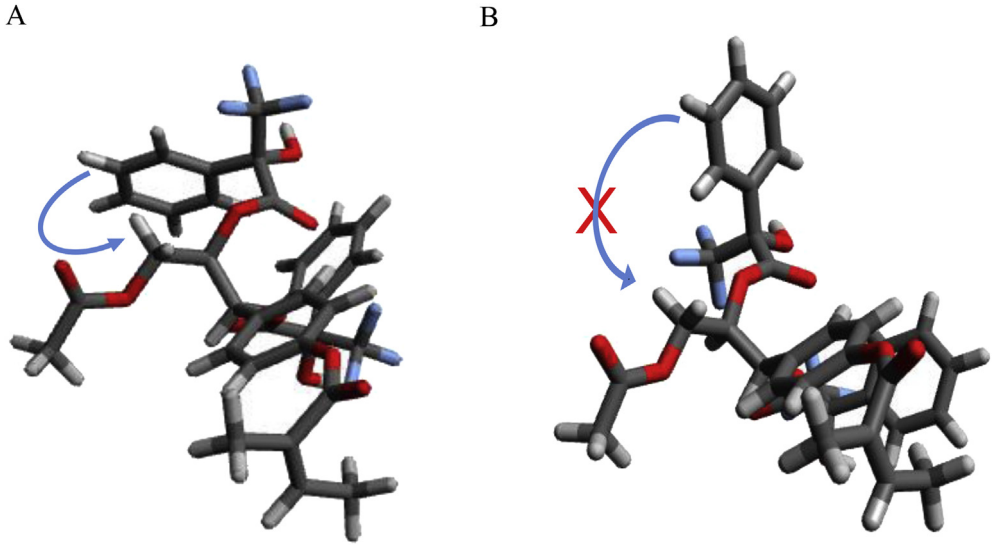

Fig. 2. Most stable conformer of A) di-(*S*)-MTPA-**1** and B) di-(*R*)-MTPA-**1**. Arrow indicates the effect of phenyl group on C9 protons.

that epoxide **2** is a natural product.

Compound **3**, with a molecular formula of C_{16} H_{20} O_6 (obs. *m/z* 331.11630 for C_{16} H_{20} NaO_6, calcd *m/z* 331.11521), was deduced, based on 1D- and 2D-NMR data, to also be a phenylpropanoid that was almost identical to **1**, differing in the chemical shift of C-7 [δ_C 61.86] and the coupling constant of H-7, $J = 7$ Hz, suggesting that this compound is the *threo* isomer. The negative Cotton effect observed in the CD spectrum for this compound suggested the *R*-configuration to C-7 (Seco et al., 2001) and according to the *threo* configuration, it is suggested that **3** has a 7*R*,8*R* configuration. This compound was present the amount of 46 mg/100 g of dried plant material.

To the best of our knowledge, these three phenylpropanoids have not been previously described and herein the name of them are proposed for charuol A (**1**), charuepoxide (**2**) and charuol B (**3**).

The phytotoxic activities of compounds **1**–**3** were evaluated on *P. miliaceum* and *R. sativus* seeds as suitable models of mono- and dicotyledonous species because of their high germination rate and small size. The effective concentrations for 50% inhibition of germination (ECg_{50}) and 50% inhibition of elongation of root (ECr_{50}) and shoot (ECs_{50}) tissues were determined for both test species (Table 2). Compound **1** was the most active inhibitor of germination of *P. miliaceum*, similar to dicamba, a synthetic broadleaf auxinic herbicide, which showed an ECg_{50} of 0.99 mM (Fig. 3). In addition, **1** was the most effective of the phenylpropanoids at regulating the growth of *P. miliaceum* seedlings (Table 2).

Compound **1** was also the most active compound capable of inhibiting the germination and seedling growth of *R. sativus* (Table 2, Fig. 3) with a similar potency to that of dicamba, although the latter was more effective than **1** inhibiting the growth of *R. sativus*. The seedling development of *R. sativus* was affected identically by **2** and by **3**.

Compounds **1** and **3** showed similar growth inhibition activity of shoot and root against *R. sativus* and of root against *P. miliaceum* while **2** with an epoxide in C7-C8 was the less active. These results suggest that the free hydroxyls are a requirement for phytotoxicity. However, the spatial arrangement of the hydroxyl groups is likely involved in germination inhibition because **1** was more active than

Table 2
Inhibition of germination and seedling growth of the isolated compounds against *Panicum miliaceum* and *Raphanus sativus*.

	Raphanus sativus			*Panicum miliaceum*		
	Values expressed in mM (confidence interval)					
	ECg_{50}	ECs_{50}	ECr_{50}	ECg_{50}	ECs_{50}	ECr_{50}
1	1.94 (0.86–3.02)	1.62 (0.42–2.82)	2.6 (1.37–3.83)	0.97 (0.47–1.47)	1.00 (0.36–1.65)	1.59 (1.24–1.94)
2	9.98 (8.95–11.01)	15.1 (6.32–23.88)	6.88 (4.64–9.12)	3.78 (1.54–6.02)	4.82 (1.40–8.24)	2.06 (1.56–2.56)
3	10.00 (4.9–15.10)	1.78 (1.02–2.54)	2.95 (2.39–3.51)	2.69 (1.69–3.69)	3.21 (2.34–4.08)	1.00 (0.73–1.27)
dicamba	2.98 (2.33–3.64)	0.04 (0.021–0.053)	1.310^{-4} (910^{-6}–210^{-4})	0.99 (0.36–1.62)	2.5 (1.55–3.45)	4×10^{-3} (1×10^{-3}–7×10^{-3})

3 in both species. The most active compound **1** was also assayed against another 4 species (Macías et al., 2000a, b) and the results are shown in Table 3 and Fig. 3. The most sensitive species was *Lactuca sativa* (lettuce), followed by *Eruca sativa* (rocket) and *Allium amprelopraysum* (leek) with EC_{50} ranging 90–260 μM for germination inhibition and 110–300 μM for the growth inhibition. The phenylpropanoid **1** inhibitory effect on root growth of *L. sativa* was of the same order of magnitude as some steroidal saponins isolated from *Agave offoyana* leaves (Pérez et al., 2014) and as other plant secondary metabolites (D'Abrosca et al., 2005; del Corral et al., 2012; Macías et al., 2000a, b). The results on germination and seedling growth inhibition of the test species showed that **1** affects both mono- and dicotyledonous species with similar potency.

As hundreds of weed species have developed resistance to commercial herbicides (Heap, 2016), new ecofriendly management systems are urgently required, in order to reduce dependence on synthetic agrochemicals. Phytotoxins produced by plants are widespread in nature affecting the growth and reproduction of other species, in consequence they may reasonably be proposed for weed management especially in organic farming. Knowledge of the active species native to a given region helps farmers to use local plants to control weeds in their agricultural productions. This practice may avoid transport and other contributions to global warming. The new phenylpropanoids isolated here are good examples of phytotoxins which can be obtained from a widely distributed native plant and thus contribute to more environmentally sound agricultural practices.

2.1. Conclusion

In summary, three novel phenylpropanoids, (**1**, **2** and **3**) were isolated from *O. charua*. They showed phytotoxic activity against mono- and dicotyledonous species, suggesting that they could reasonably be used for weed management in more ecofriendly agricultural practices.

3. Experimental

3.1. General experimental procedures

IR spectra were obtained using KBr disks on a Shimadzu spectrophotometer. ^{1}H- and ^{13}C-NMR spectra, including COSY, HMBC, HSQC and ROESY experiments, were recorded in $CDCl_3$ with a Bruker AVANCE II 400 spectrometer (Bruker, Germany) operated at 400 MHz for ^{1}H and at 100 MHz for the ^{13}C nucleus. Chemical shifts (parts per million) are relative to internal tetramethylsilane (TMS) used as a reference ($\delta = 0.00$). UV spectra were recorded on a Hewlett Packard 8452 A diode array spectrophotometer. CD spectra were obtained on a J-800 spectropolarimeter (JASCO Co., Japan). Optical rotation angles were measured in a JASCO DIP-370 spectropolarimeter (JASCO Co., Japan). High resolution mass spectra were determined in a Bruker MicroQTOF-QII mass spectrometer (Bruker Daltonics, USA), equipped with an ESI source operated in positive mode at 200 °C with a capillary voltage of 4500 V. Mass accuracy was verified by calibration before and after sample introduction, using sodium formate (100 mM). Both samples and calibrant were introduced using a syringe pump at 3 μL min^{-1}. HPLC (Shimadzu, Japan) was performed on a Phenomenex Prodigy 5μ ODS (4.6 mm i.d. × 250 mm) reversed-phase column eluted with CH_3CN/H_2O (45:55) as mobile phase, flow rate 0.8 ml/min and UV detection at 210 nm for **1**, **2** and **3**.

3.2. Plant material

Aerial parts of *O. charua* were collected in the hills of Córdoba Province, Argentina (31°35′32,06″ S, 64°31′38.72″ W), in December 2013. A voucher specimen (UCCOR 175) is deposited in the "Marcelino Sayago" Herbarium of the School of Agricultural Science, Catholic University of Córdoba and was authenticated by the botanist, Gustavo Ruiz.

3.3. Seeds

Proso millet (*Panicum miliaceum*), radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), rocket (*Eruca sativa*), leek (*Allium ampeloprasum*) and rye (*Secale cereale*) seeds used for the phytotoxicity assays were purchased from Semillería Florensa in Córdoba, Argentina.

3.4. Germination bioassay

Ten seeds of either *L. sativa*, *E. sativa* or *A. ampeloprasum*; fourteen seeds of *P. miliaceum*, and six seeds of *R. sativus* or *S. cereale* were placed in a 12-well multiwell plate lined with filter paper, treated with chromatographic fractions (300 μl, see section 3.5) at 5, 2.5, 1.25, 0.62, 0.31 mg ml^{-1} or solutions of pure compound **1**, **2** or **3** (all in water-acetone 3%) 5 to 0.018 mg ml^{-1}. The herbicide dicamba (98% purity, a gift from Lanther Química, Argentina) was likewise assayed after dissolving in H_2O at 5, 2.5, 1.25, 0.62, 0.31, 0.15, 0.075, 0.037, 0.018, 0.009, 0.004, 0.002, 0.001, 0.0005 mg ml^{-1}. Controls received H_2O-acetone (300 μl, 97:3 v/v). Each treatment was replicated three times. The dishes were covered with aluminum foil and placed in a growth chamber (25 ± 1 °C, 70–75% relative humidity, with a photoperiod of 16:8 light-dark cycle).

After 2–7 days depending on the test seed, germination was assessed and the number of germinated seeds and the lengths of seedling roots and shoots were recorded. Germination and root and shoot length inhibition indices were calculated as IG, IR and IS % = [1- (T/C)] x 100, where T and C are either the number of seeds germinated or the root or shoot length in the treatment and the control, respectively. Effective dose 50 values for germination and for root and shoot growth inhibition were calculated as ECg_{50}, ECr_{50} and ECs_{50}, respectively. The results were analyzed by *t*-test ($p < 0.05$) and ECg_{50}, ECr_{50} and ECs_{50} values were calculated by Probit analysis based on the percentage of the inhibition of germination (IG%) and the inhibition of root (IR%) and shoot growth (IS%), respectively.

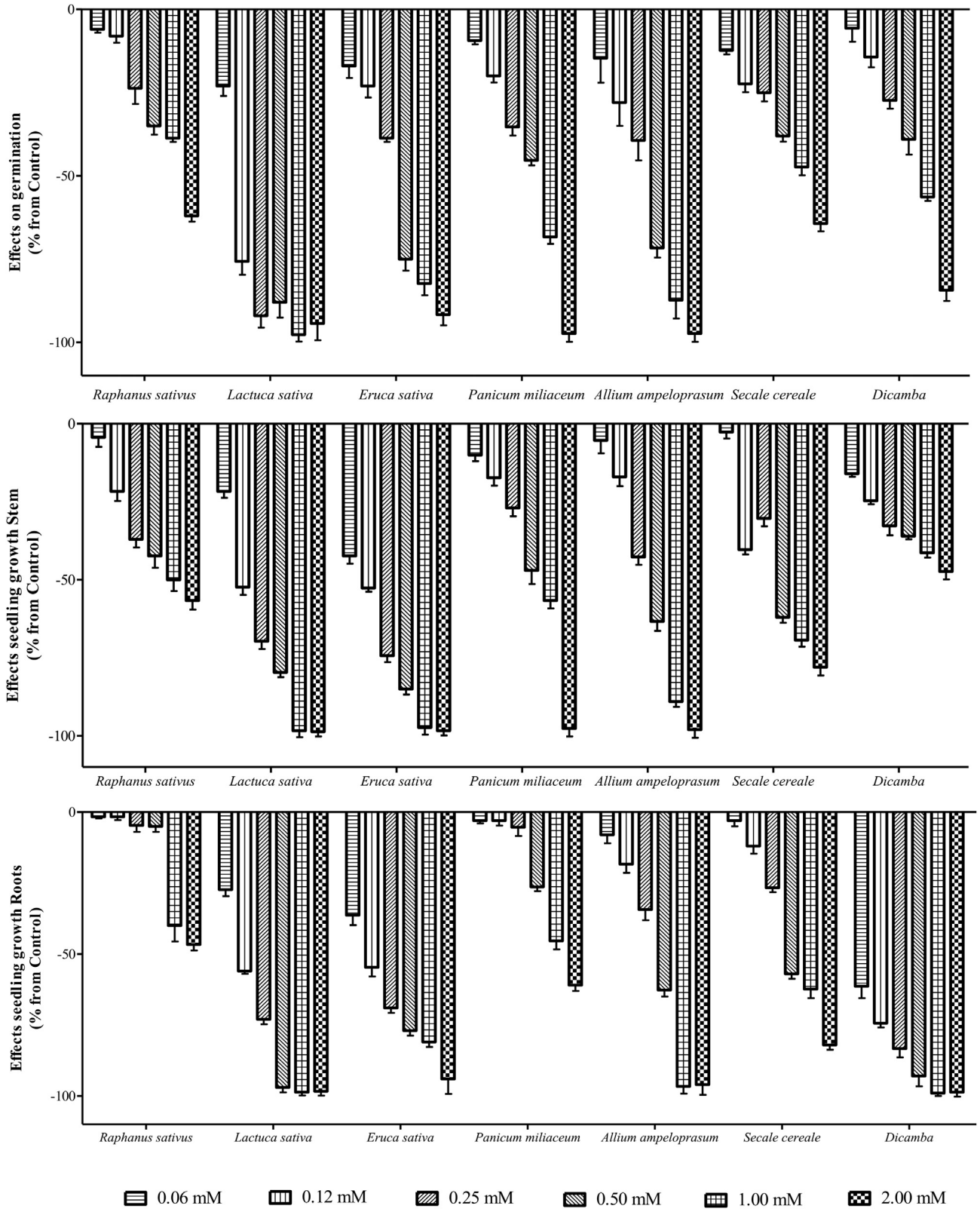

Fig. 3. Effects of **1** on germination and growth development of different test species and germination and growth inhibition of dicamba against *Panicum miliaceum.*

3.5. Extraction and bio-guided isolation

The vegetable material of *O. charua* was air-dried at room temperature, crushed, and extracted (208.8 g) by 48 h maceration with EtOH (1.5 L). The EtOH extract was concentrated in vacuo to yield the crude material (21.3 g). The latter was partitioned between H_2O (500 ml) and Et_2O (3 × 250 ml), yielding aqueous and ether fractions, representing 19% and 81% of the original extract,

Table 3
Inhibition of germination and seedling growth of several species by compound **1**.

Species	Values expressed in mM (confidence interval)		
	ECg_{50}	ECs_{50}	ECr_{50}
Lactuca sativa	0.09 (0.085–0.095)	0.14 (0.13–0.15)	0.12 (0.11–0.13)
Eruca sativa	0.19 (0.14–0.23)	0.11 (0.050–0.17)	0.12 (0.08–0.16)
Allium ampeloprasum	0.26 (0.24–0.28)	0.30 (0.28–0.32)	0.30 (0.24–0.36)
Secale cereale	1.07 (0.78–1.36)	0.53 (0.34–0.72)	0.67 (0.41–0.93)

respectively. The germination inhibition indices (IG%) against *R. sativus* for the aqueous and ether fractions were 0 and 100, respectively. A portion of the Et_2O fraction (6.2 g) was fractionated by silica gel column chromatography (cc) eluted with a gradient of hexane/Et_2O. Based on TLC analyzed with UV-light 254 nm, the fractions with similar compound profiles were pooled, resulting in a total of six fractions, 1–6. The active fraction 2 (190 mg; IG % = 94 at 5 mg ml^{-1}) was then subjected to circular preparative thin layer chromatography with hexane/Et_2O in a gradient (1:0–0:1) to give fractions 1–29 on the basis of TLC analysis. Fraction 9 showed IG% = 96 against *R. sativus* and was further fractionated by silica gel cc eluted with hexane:Et_2O (8:2) affording compound **2** (9 mg). Fractions 17 and 28 were also each further purified by separate silica gel cc eluted with hexane:Et_2O (7:3), yielding compounds **3** (24 mg) and **1** (31 mg), respectively.

3.6. Compound characterization

(*Z*)-4-((1*S*,2*R*)-3-acetoxy-1,2-dihydroxypropyl)phenyl 2-methylbut-2-enoate (**1**): Colorless oil; $[\alpha]^{22}{}_{D}$ −0.10 (*c* 0.18 %m/v, MeOH); UV (CH_3CN) λ_{max} (log ε):220 (1.72) nm; CD (CH_3CN) λ_{max} ($\Delta\varepsilon$): 250 (+0.028), 261 (+0.017), 268 (+0.014); IR (KBr) ν_{max} 3475.55, 2964.43, 1736.32, 1722.13, 1505.61 cm^{-1}; For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Table 1; HR-TOF-MS (ESI positive) *m/z* 331.11521 $[M + Na]^+$ (calcd for $C_{16}H_{20}NaO_6$, 331.11546).

(*Z*)-4-((2*S*,3*R*)-3-(acetoxymethyl oxiran-2-yl)phenyl 2-methylbut-2-enoate (**2**): Colorless oil; $[\alpha]^{21}{}_{D}$ −0.14 (*c* 0.8 %m/v, MeOH); UV (CH_3CN) λ_{max} (log ε): 222 (1.79) nm; CD (CH_3CN) λ_{max} ($\Delta\varepsilon$): 251.0 (+0.023), 263.0 (+0.020), 269 (+0.014); IR (KBr) ν_{max} 3521.69, 2960.88, 1739.87, 1722.13, 1517.71 cm^{-1}; For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Table 1; HR-TOF-MS (ESI positive) *m/z* 313.10541 $[M + Na]^+$, (calcd for $C_{16}H_{18}NaO_5$, 313.10464).

(*Z*)-4-((1*R*,2*R*)-3-acetoxy-1,2-dihydroxypropyl)phenyl 2-methylbut-2-enoate (**3**): Colorless oil; $[\alpha]^{22}{}_{D}$ −0.20° (*c* 0.2 %m/v, MeOH); UV (CH_3CN) λ_{max} (log ε) 224 nm (63.64); CD (CH_3CN) λ_{max} ($\Delta\varepsilon$): 254 (−0.0018), 263.0 (-0.0018), 271.0 (-0.0082) IR (KBr) ν_{max} 3489.74, 2964.43, 1736.32, 1722.13, 1512.71 cm^{-1}; ^1H NMR ($DCCL_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Table 1; HR-TOF-MS (ESI positive) *m/z* 331.11521 $[M + Na]^+$ (calcd for $C_{16}H_{20}NaO_6$ 331.11630).

3.7. Preparation of (R)- and (S)-MTPA ester of compound 1

Compound **1** (7 mg) was treated with anhydrous CH_2Cl_2 (5 ml) solutions of Et_3N (61 mg), *N*,*N*-dimethylaminopyridine (6.1 mg) and (*S*)- [or (*R*)-] 2-methoxy-2- trifluoromethyl-2-phenylacetyl chloride (63 mg in 2.5 ml) (Velten et al., 1994). The mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure and the residue was applied to silica gel cc eluted with hexane:ether (7:3). This process afforded 5 mg of either the di-(*R*)-MTPA ester **1** or 6 mg of di-(*S*)-MTPA ester **1**. di-(*R*)-MTPA ester **1**: colorless oil, ^1H NMR (400 MHz, $CDCl_3$): 2.00 (3H, s, H-C2′), 2.04 (3H, t, *J* = 1.6 Hz, *J* = *2.8* Hz, H-C4″), 2.08 (3H, dd, *J* = 1.6 Hz, *J* = 7.2 Hz, H-C5″), 3.42 (3H, d, *J* = 1.8 Hz, H-C9b‴), 3.47 (3H, d, *J* = 1.2 Hz, H-C3b‴), 4.10 (1H, dd, *J* = 7.6 Hz, *J* = 12 Hz, H-C9a), 4.35 (1H, dd, *J* = 2.8 Hz, *J* = 12 Hz, H-C9b), 5.64 (1H, m, H-C8), 6.18 (1H, d, *J* = 4.8 Hz, H-C7), 6.28 (1H, dd, *J* = 1.6 Hz, *J* = 7.2 Hz, H-C3″), 6.99 (1H, d, *J* = 8 Hz, H-C3), 6.99 (1H, d, *J* = 8 Hz, H-C5), 7.07 (1H, d, *J* = 8 Hz, H-C2), 7.07 (1H, d, *J* = 8 Hz, H-C6), 7.30 (1H, d, *J* = 8 Hz, H-C4a‴), 7.30 (1H, d, *J* = 8 Hz, H-C8a‴), 7.30 (1H, d, *J* = 8 Hz, H-C4b‴), 7.30 (1H, d, *J* = 8 Hz, H-C8b‴), 7.34 (1H, s, H-C6a‴), 7.34 (1H, s, H-C6b‴), 7.42 (1H, d, *J* = 6 Hz, H-C5a‴), 7.42 (1H, d, *J* = 6 Hz, H-C7a‴), 7.42 (1H, d, *J* = 6 Hz, H-C5b‴), 7.42 (1H, d, *J* = 6 Hz, H-C7b‴). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.01 (C-5″), 20.47 (C-2′), 20.58 (C-4″), 55.50 (C-9a‴), 55.70 (C-9b‴), 61.00 (C-9), 74.48 (C-8), 74.68 (C-7), 84.45 (C-2a‴), 84.70 (C-2b‴), 122.11 (C-3), 122.11 (C-5), 123.57 (C-10a‴), 123.57 (C-10b‴), 126.94 (C-2″), 127.12 (C-4a‴), 127.12 (C-8a‴), 127.12 (C-4b‴), 127.12 (C-8b‴), 128.17 (C-2), 128.17 (C-6), 128.44 (C-5a‴), 128.44 (C-7a‴), 128.44 (C-5b‴), 128.44 (C-7b‴), 128.46 (C-6a‴), 128.46 (C-6b‴), 129.70 (C-3a‴), 129.70 (C-3b‴), 130.90 (C-1), 141.07 (C-3″), 151.30 (C-4), 164.93 (C-1″), 165.66 (C-1a‴), 165.97 (C-1b‴), 170.05 (C-1′).

HR-TOF-MS (ESI positive) *m/z* 763.19320 $[M + Na]^+$, (calcd for $C_{36}H_{34}F_6NaO_{10}$, 763.19784).

di-(*S*)-MTPA ester **1**: colorless oil, ^1H NMR (400 MHz, $CDCl_3$): 1.96 (3H, s, H-C2′), 2.05 (3H, t, *J* = 1.2 Hz, *J* = 2.8 Hz, H-C4″), 2.09 (3H, dd, *J* = 2.0 Hz, *J* = 8.0 Hz, H-C5″), 3.25 (3H, d, *J* = 1.0 Hz, H-C9b‴), 3.37 (1H, d, *J* = 1 Hz H-C9a‴), 3.80 (1 H, dd, *J* = 6 Hz, *J* = 12.5 Hz, H-C9a), 4.31 (1 H, dd, *J* = 2.8 Hz, *J* = 12.5 Hz, H-C9b), 5.61 (1 H, m, H-C8), 6.24 (1H, d, *J* = 6.8 Hz, H-C7), 6.30 (1H, dd, *J* = 1.6 Hz, *J* = 7.3 Hz, H-C3″), 7.14 (1H, d, *J* = 8.6 Hz, H-C3), 7.14 (1H, d, *J* = 8.6 Hz, H-C5), 7.21 (1H, d, *J* = 8.0 Hz, H-C4a‴), 7.21 (1H, d, *J* = 8.0 Hz, H-C8a‴), 7.21 (1H, d, *J* = 8.0 Hz, H-C4b‴), 7.21 (1H, d, *J* = 8.0 Hz, H-C7b‴), 7.31 (1H, d, *J* = 8.0 Hz, H-C5a‴), 7.31 (1H, d, *J* = 8.0 Hz, H-C7a‴), 7.31 (1H, d, *J* = 8.0 Hz, H-C5b‴), 7.31 (1H, d, *J* = 8.0 Hz, H-C7b‴), 7.34 (1H, s, H-C6a‴), 7.34 (1H, s, H-C6b‴), 7.39 (1H, d, *J* = 8.6 Hz, H-C2), 7.39 (1H, d, *J* = 8.6 Hz, H-C6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.06 (C-5″), 20.39 (C-2′), 20.60 (C-4″), 55.26 (C-9a‴), 55.51 (C-9b‴), 60.90 (C-9), 73.83 (C-8), 73.95 (C-7), 84.29 (C-2a‴), 84.70 (C-2b‴), 122.38 (C-3), 122.38 (C-5), 123.12 (C-10a‴), 123.12 (C-10b‴), 126.92 (C-2″), 127.08 (C-5a‴), 127.08 (C-7a‴), 127.08 (C-5b‴), 127.08 (C-7b‴), 128.38 (C-4a‴), 128.38 (C-8a‴), 128.38 (C-4b‴), 128.38 (C-8b‴), 129.08 (C-2), 129.08 (C-6), 129.58 (C-6a‴), 129.58 (C-6b‴), 131.47 (C-1), 131.71 (C-3a‴), 131.74 (C-3b‴), 141.25 (C-3″), 151.63 (C-4), 165.32 (C-1″), 165.65 (C-1a‴), 165.73 (C-1b‴), 170.02 (C-1′). HR-TOF-MS (ESI positive) *m/z* 763.19647 $[M + Na]^+$, (calcd for $C_{36}H_{34}F_6NaO_{10}$, 763.19784).

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