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Original Research Paper

Weeds as biocatalysts in the stereoselective synthesis of chiral phenylethanols used as key intermediates for pharmaceuticals

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ABSTRACT

This paper describes the search for novel vegetal biocatalysts for the stereoselective reduction of prochiral phenylketones. In this study, twenty native weeds were tested and *Eryngium horridum* Malme (Apiaceae) was proven to be an effective biocatalyst for the stereoselective reduction of acetophenone to (*S*)-1-phenylethanol (96% conversion, > 99.9 e.e.%). Using this biocatalyst, fourteen chiral (*S*)-phenylethanols with excellent enantiomeric excesses (> 98%) and variable conversions (30–100%) were obtained.

1. Introduction

The molecular complexity of currently used chemical compounds is rising and is characterized by a growing number of chiral centers. Moreover, because of safety and therapeutic and regulatory concerns, there has been an increasing interest in the development of processes capable of producing enantiomerically pure drugs (Hutt and Tan 1996; Rouhi 2003). Thus, it follow that the stereoselective production of enantiomerically pure molecules is the most critical step in the preparation of chiral building blocks for the pharmaceutical industry (Food and Drug Administration, 1992).

The asymmetric reduction of prochiral ketones represents a pivotal transformation in organic synthesis and can be performed using different catalytic processes (Singh, 1992). However, biocatalytic reduction provides an attractive means to reduce stereoselectively a broad range of ketones because of some comparative advantages (Wohlgemuth, 2010). Favorable characteristics of biocatalysts include their low cost, high versatility and efficiency, in addition to highly desirable chemical aspects such as chemoselectivity, regioselectivity and enantioselectivity. Moreover, there is the added advantage of using reagents for organic transformations that can be used on a sustainable basis, rather than depleting resources (Cordell et al., 2007). For these reasons, over the past decade the application of biocatalytic processes in the commercial synthesis of chiral alcohols has undergone a revolution. Biocatalysts are now often the preferred catalyst for the synthesis of chiral alcohols via ketone reduction (Huisman et al., 2010).

In recent years, chemical reactions using parts of plants as biocatalysts have received great attention because of their many advantages (Cordell et al., 2007). First of all, a large array of taxonomically different plants is available at a very low cost with these systems also having the advantage of being environmentally friendly due to the reaction being carried out in water as the solvent, and because the catalyst is biodegradable. For this reason, these processes generate less waste than conventional chemical reagents (Kumaraswamy and Ramesh, 2003).

In this sense, many transformations of different substrates, such as hydroxylation and oxidation reactions (*Gynostemma*

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pentaphyllum, Sakamaki et al., 2005), hydrolysis of esters (*Solanum tuberosum, Helianthus tuberosus*, Mironowicz, 1998), bioreduction of ketones and aldehydes (*Daucus carota, Foeniculum vulgare, Cucurbita pepo, Phaseolus aureus, Cocos nucifera, Saccharum officinarum, Manihot dulcis, Manihot esculenta, Mespilus germanica, Citrus reticulata,* Cordell et al., 2007; Yadav et al., 2002; Bruni et al., 2006; Villa et al., 1998; Kumaraswamy and Ramesh, 2003; Fonseca et al., 2009; Baldassarre et al., 2000; Maczka and Mironowicz, 2004; Machado et al., 2006, 2008; Assunção et al., 2008; Blanchard and van de Weghe, 2006; Bennamane et al., 2014, 2015), enzymatic lactonization (*Malus sylvestris, Helianthus tuberosus*, Olejniczak et al., 2003), glycosylation (*Ipomoea batatas, Eucalyptus perriniana*, Shimoda et al., 2008), etc., have been performed using plants as biocatalysts, and have produced very good results. Moreover, the use of functionally intact cells ("whole plant cells") obtained directly from cut portions of plants have emerged, because the whole cells also ensure the recycling of the oxidized cofactors (Blanchard and van de Weghe, 2006). Additionally, these reaction systems do not need laborious cultivation or development operations to be performed, which are commonly employed in the management of microorganisms (Bohman et al., 2009).

This new methodology recently has been named as "*Botanochemistry*" and offers numerous advantages in terms of biodisponibility and economy of time, since fastidious steps of preparation, extraction, purification and multiplication of the biocatalyst are not necessary, thus promoting the preservation of a maximum catalytic activity of the enzymes (Vandenberghe et al., 2013). Additionally, botanochemistry is an interesting way to promote by-products of agriculture such as vegetable peelings that cannot be used for human consumption by food companies or for the use of those vegetable species which do not have any other reported practical utility and are simply considered to be weeds.

Recently, a project was commenced with the aim of identifying green procedures to obtain chemical intermediates using plants as biocatalyts. With this objective in mind, the screening of the some native weeds was initiated to search for plants that could be used as biocatalysts in the reduction of prochiral ketones in order to obtain chiral phenylethanols. In the particular case of these chiral alcohols, several were considered as key starting materials in the synthesis of scented substances for the pharmaceutical industries (Huisman et al., 2010). For this reason, this work is focused on the use of plants which are considered to be weeds for the stereoselective reduction of phenylketones as a sustainable alternative to traditional chemical methods.

2. Experimental

2.1. Generals

Ketones and $NaBH_4$ were purchased from Sigma-Aldrich S.A. (Argentina). Sterile deionizated water was used in all experiences. Ethyl acetate and hexane were purified by a simple distillation. GC analyses were made on a Shimadzu GC-14B instrument with a FID detector, and GC–MS analyses were carried out on a gas chromatograph Hewlett Packard HP 5890 Series II equipped with the Mass Detector HP 5970. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 MHz using $CDCl_3$ as the solvent, and optical rotations were measured in a JASCO P-1010 polarimeter.

2.2. Biocatalysts

Healthy plants were collected in the Punilla Valley (Province of Córdoba, Argentina) and were identified by a botanist. Those selected had roots similar in form and texture to those a carrot (model vegetal biocatalyst) (Yadav et al., 2002). The aerial parts were discarded, and the roots were extensively washed with tap water to remove traces of soil.

2.3. General procedure for the bioreductions

Fresh plant roots were maintained in a 5% sodium hypochlorite aqueous solution for 10–20 min. Thus, they were washed with sterile deionized water again and the external layer was removed, with the remaining roots being cut into small thin slices (1 cm) with a sterile cutter. Treated and cut roots (10 g) were added to a sterile Erlenmeyer flask (250 mL) with sterile deionized water (75 mL), and ketone (50 mg) was added to this suspension. The reaction was carried out by stirring on an orbital shaker (120 rpm) at room temperature with the Erlenmeyer flask being closed. Then, the crude reaction was filtered through cotton, and the solution was extracted with ethyl acetate (3×40 mL). Finally, anhydrous calcium chloride was added to remove the dissolved water, and the organic solution was filtered and analyzed (1 μL) by chiral GC-FID and GC–MS. Thus, the organic solutions were evaporated, and the products were filtered on a short column with silica gel (70–230 mesh) using hexane-ethyl acetate in variable proportions as the eluent; the isolated yield was determined and the structure of the products was corroborated by ^1H NMR and ^{13}C NMR. Similarly, a control experiment was conducted without the addition of the corresponding phenylketone, and the crude reaction was analyzed by chiral GC-FID and GC–MS analyses.

2.4. Kinetic study of the bioreduction of acetophenone

In order to establish the optimal reaction time using *E. horridum* as biocatalyst, a kinetic study was made using acetophenone as the model substrat. The reaction progress was monitored by taking samples (2 mL) every 24 h, which were first extracted by shaking with ethyl acetate (2 mL) and the organic layer was collected. Then, anhydrous calcium chloride was added to remove the dissolved water, and the organic solution was filtered and analyzed (2 μL) by GC using the same general conditions described in GC-FID and GC–MS analyses. The reactions were made in triplicate.

2.5. GC-FID and GC–MS analyses

To establish the chromatographic conditions, all substrates used were previously reduced with $NaBH_4$ (Sigma-Aldrich S.A. Argentina) in ethanol to obtain the racemic mixture. The GC separations were performed on a fused silica capillary column Supelco β-Dex120 (phenyl-polysiloxane with 20% of permethylated β-Cyclodextrine, 30 m, 0.25 mm, 0.25 μm film thickness) with general GC conditions of: split mode 1/50; injector 220 °C; detector FID 220 °C; carrier gas N_2; head pressure 100 kPa. The conversion percentages of the reactions were determined by GC using the normalized peak areas without a correction factor, and the GC–MS (70 eV) analyses were performed using the same conditions as those in the GC analysis, but using a capillary column Hewlett Packard HP-5 (Crosslinked 5% PhMe Siloxane, 30 m, 0.3 mm, 0.25 μm film thickness).

2.6. GC and spectroscopic data

All products were identified by comparison of their GC retention times, MS, ^1H and ^{13}C NMR spectra with literature data. (Salvi and Chattopadhyay, 2001, 2008; Yu et al., 2011; Cheemala et al., 2007).

2.6.1. (−)-(S)-1-Phenylethanol

GC conditions: $T_1 = 80$ °C (1 min), $\Delta T = 2.5$ °C/min, $T_2 = 140$ °C. GC Rt acetophenone: 16.15 min, Rt (+)-(*R*)-1-phenylethanol:

20.88 min and Rt (−)-(*S*)-1-phenylethanol: 21.58 min. Yield: 29 mg (58%). $[\alpha]_D^{23} = -43.1$, $c=0.55$ MeOH (Yadav et al. 2002, $[\alpha]_D^{25} = -39.1$, $c=3.5$ MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)= 1.49 (d, 3H, CH_3), 2.03 (s, 1H, OH), 4.89 (q, 1H, CH), 7.25–7.39 (m, 5H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=25.16, 70.44, 125.39, 127.50, 128.52, 145.81. EI MS *m/z* (rel. int. %)=123 (M^++1, 4), 122(M^+, 29), 121 (M^+-1, 6), 107 (74), 105 (13), 79 (100), 78 (26), 77 (68), 57 (19), 51 (30).

2.6.2. (−)-(S)-1-(2′-Chlorophenyl)ethanol

GC conditions: T_1=110° C, ΔT=3 °C/min, T_2=210 °C. GC Rt 2′-chloroacetophenone: 10.88 min, Rt (+)-(*R*)-1-(2′-chlorophenyl) ethanol: 17.04 min and Rt (−)-(*S*)-1-(2′-chlorophenyl)ethanol: 18.30 min. Yield: 26 mg (52%). $[\alpha]_D^{23} = -60.7$, $c=0.55$ MeOH (Nakamura and Matsuda, 1998, $[\alpha]_D^{25} = -62.7$, c=0,894 $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)= 1.50 (d, 3H, CH_3), 1.92 (s,1H, OH), 5.30 (c, 1H, CH), 7.18–7.24 (m, 1H, ArH), 7.28–7.34 (m, 2H, ArH), 7.59–7.61 (m, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=23.52, 67.00, 126.41, 127.22, 128.42, 129.42, 131.67, 143.04. EI MS *m/z* (rel. int. %)=158 (M^++2, 5), 156(M^+, 15), 143 (24), 141 (82), 113 (26), 77 (100), 51 (28).

2.6.3. (−)-(S)-1-(3′-Chlorophenyl)ethanol

GC conditions: T_1=110 °C (1 min), ΔT=3 °C/min, T_2=210 °C. GC Rt 3′-chloroacetophenone: 12.94 min, Rt (+)-(*R*)-1-(3′-chlorophenyl)ethanol: 19.60 min and Rt (−)-(*S*)-1-(3′-chlorophenyl) ethanol: 20.14 min. Yield: 25 mg (50%). $[\alpha]_D^{23}$ = -40.2, c = 0.54 MeOH (Nakamura and Matsuda, 1998, $[\alpha]_D^{25}$ = −43.5, c =1.08, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 1.48 (d, 3H, CH_3), 1.89 (d, 1H, OH), 4.85–4.90 (m, 1H, CH), 7.22-7.30 (m, 3 H), 7.36-7.39 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 25.25, 69.83, 123.54, 125.64, 127.55, 129.80, 134.39, 147.86. EI MS *m/z* (rel. int. %) = 158 (M^++2, 5), 156 (M^+, 16), 143 (16), 141 (54), 121 (11), 115 (11), 113 (38), 77 (100), 75(17), 51 (23).

2.6.4. (−)-(S)-1-(4′-Chlorophenyl)ethanol

GC conditions: T_1=110 °C, ΔT=3 °C/min, T_2=210°C (20 min). GC Rt 4′-chloroacetophenone: 13.26 min, Rt (+)-(*R*)-1-(4′-chlorophenyl)ethanol: 18.09 min and Rt (−)-(*S*)-1-(4′-chlorophenyl) ethanol: 18.65 min. Yield: 28 mg (56%). $[\alpha]_D^{23} = -44.3$, $c=0.40$ MeOH (Nakamura and Matsuda, 1998, $[\alpha]_D^{25} = -49.0$, $c=1.84$, Ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.47 (d, 3H, CH_3), 1.83 (s, 1H, OH), 4.87 (q, 1H, CH), 7.26–7.31 (m, 4H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=25.29, 69.77, 126.80, 128,62, 133.09, 144.25. EI MS *m/z* (rel. int. %)=158 (M^++2, 4), 156 (M^+, 16), 143 (20), 141 (70), 121 (9), 113 (28), 77 (100), 75 (19), 51 (24).

2.6.5. (+)-(S)-1-(2′-Nitrophenyl)ethanol

GC conditions: T_1=100 °C, ΔT=2 °C/min, T_2=210 °C (20 min). GC Rt 2′-nitroroacetophenone: 31.15 min, Rt (−)-(*R*)-1-(2′ nitrophenyl)ethanol: 40.48 min and Rt (+)-(*S*)-1-(2′-nitrophenyl) ethanol: 42.23 min. Yield: 11 mg (22%). $[\alpha]_D^{23} = +20.0$, $c=0.018$ MeOH (Comasseto et al., 2006, $[\alpha]_D^{25} = +30.6$, $c=3.06$ $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.56 (d, 3H, CH_3), 2.41 (b, 1H, OH), 5.40 (c, CH), 7.40 (dt, 1H, ArH), 7.63 (dt, 1H, ArH), 7.82 (dd, 1H, ArH), 7.88 (dd, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)= 24.20, 65.59, 124.30, 127.58, 128.11, 133.59, 140.90, 147.89. EI MS *m/z* (rel. int. %)=165 (M^+ −2, 1), 150 (100), 123 (12), 104 (7), 91 (11), 77 (14), 76 (35), 74 (14), 63 (11), 51 (50).

2.6.6. (−)-(S)-1-(3′-Nitrophenyl)ethanol

GC conditions: T_1=100 °C, ΔT=2 °C/min, T_2=210 °C (20 min). GC Rt 3′-nitroroacetophenone: 34.18 min, Rt (+)-(*R*)-1-(3′-nitrophenyl)ethanol: 45.97 min and Rt (−)-(*S*)-1-(3′-nitrophenyl) ethanol: 46.35 min. Yield: 13.5 mg (27%). $[\alpha]_D^{23} = -25.8$, $c=0.50$ MeOH (Comasseto et al., 2006, $[\alpha]_D^{25} = -30.5$, $c=2.99$ $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.54 (d, 3H, CH_3), 2.17 (b, 1H, OH), 5.02 (c, 1H, CH), 7.50-7.54 (t, 1H, ArH), 7.71–7.73 (d, 1H, ArH), 8.11–8.14 (dm, 1H, ArH), 8.27 (t, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=25.49, 69.39, 120.45, 122.37, 129.44, 131.57, 147.91, 148.42. EI MS *m/z* (rel. int. %)=167 (M^+, 1), 166 (M^+ − 1, 1), 152 (100), 121 (12), 107 (60), 106 (20), 105 (38), 78 (38), 77 (82), 76(19), 51 (32).

2.6.7. (−)-(S)-1-(4′-Nitrophenyl)ethanol

GC conditions: T_1=100 °C, ΔT=2 °C/min, T_2=210 °C (20 min). GC Rt 4′-nitroroacetophenone: 37.18 min, Rt (+)-(*R*)-1-(4′-nitrophenyl)ethanol: 48.66 min and Rt (−)-(*S*)-1-(4′-nitrophenyl) ethanol: 49.96 min. Yield: 21.5 (43%). $[\alpha]_D^{23} = -30.4$, $c=0.53$ MeOH (Yadav et al., 2002, $[\alpha]_D^{25} = -30.5$, $c=4$ $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.52 (d, 3H, CH_3), 2.05 (b, 1H, OH), 5.02 (c, 1H, CH), 7.53–7,56 (d, 2H, ArH), 8.20–8.22 (d, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=25.53, 50.88, 123.78, 126.12, 142.25, 153.04. EI MS *m/z* (rel. int. %) = 167 (M^+, 3), 166 (M^+ − 1, 1), 152 (100), 122 (13), 107 (89), 106 (22), 105 (22), 94 (31), 78(35), 77 (74), 66 (13), 51 (33).

2.6.8. (−)-(S)-1-(4′-Fluorophenyl)ethanol

GC conditions: T_1=100 °C, ΔT=2 °C/min, T_2=200 °C. GC Rt 4′-fluoroacetophenone: 9.52 min, Rt (+)-(*R*)-1-(4′-fluorophenyl) ethanol: 15.65 min and Rt (−)-(*S*)-1-(4′-fluorophenyl) ethanol: 16.54 min. Yield: 20 mg (40%). $[\alpha]_D^{23} = -34.7$, $c=0.05$ (Nakamura and Matsuda, 1998, $[\alpha]_D^{25} = -37.7$, $c=0.931$, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.47 (d, 3H, CH_3), 2.39 (b, 1H, OH), 4.87 (c, 1H, CH), 7.00–7.05 (m, 2H, ArH), 7.31–7.35 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=25.27, 69.79, 115.25 (d, J=21.2 Hz), 127.04 (d, J=8 Hz), 141.49 (d, J=3.1 Hz), 163.34 (d, J=243.8 Hz). EI MS *m/z* (rel. int. %) = 140 (M^+, 27), 125 (100), 123 (13), 97 (87), 96 (24), 95 (26), 77 (29), 75 (20), 51 (12).

2.6.9. (−)-(S)-1-(4′-Trifluorophenyl)ethanol

GC conditions: T_1=100 °C, ΔT=2 °C/min, T_2=200 °C. GC Rt 4′-trifluoroacetophenone: 8.87 min, Rt (+)-(*R*)-1-(4′-trifluorophenyl) ethanol: 16.93 min and Rt (−)-(*S*)-1-(4′-trifluorophenyl)ethanol: 18.13 min. Yield: 20.5 mg (41%). $[\alpha]_D^{23} = -25.0$, $c=0.052$ MeOH (Nakamura and Matsuda, 1998, $[\alpha]_D^{25} = -28.1$, $c=1.13$, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.51 (d, 3H, CH_3), 1.92 (b, 1H, OH), 4.97 (c, 1H, CH), 7.48–7.51(d, 2H, ArH), 7.60–7.62 (d, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=25.41, 69.83, 125.44, 125.47, 125.65, 149.71. EI MS *m/z* (rel. int. %)=190 (M^+, 13), 175 (84), 171 (11), 145 (14), 127 (100), 95 (6), 77 (12), 69 (9), 51 (10).

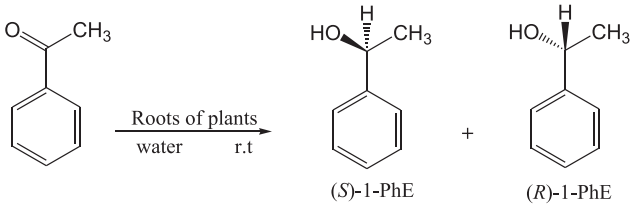
2.6.10. (−)-(S)-1-(4′-Bromophenyl)ethanol

GC conditions: T_1=100 °C, ΔT=2 °C/min, T_2=200 °C (20 min). GC Rt 4′-bromoacetophenone: 25.76 min, Rt (+)-(*R*)-1-(4′-bromophenyl)ethanol: 33.44 min and Rt (−)-(*S*)-1-(4′-bromophenyl) ethanol: 34.18 min. Yield: 21.5 (43%). $[\alpha]_D^{23} = -35.6$, $c=0.50$ MeOH (Nakamura and Matsuda, 1998, $[\alpha]_D^{25} = -37.9$, $c=1.13$, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.47 (d, 3H, CH_3), 1.92 (b, 1H, OH), 4.86 (c, 1H, CH), 7.23–7.26(m, 2H, ArH), 7.46–7.48 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) = 25.27, 69.80, 121.18, 127.16, 131.57, 144.78. EI MS *m/z* (rel. int. %) = 202 (M^++1, 27), 200 (25), 187 (81) 185 (86), 183 (9), 159 (25), 157 (31), 156 (9), 121 (23), 103 (12), 102 (8), 78 (55), 77 (100), 76 (20), 75 (21), 51 (42).

2.6.11. (−)-(S)-1-(4′-Cyanophenyl)ethanol

GC conditions: T_1=100 °C, ΔT=2 °C/min, T_2=200 °C. GC Rt 4′-cyanoacetophenone: 31.11 min, Rt (+)-(*R*)-1-(4′-cyanophenyl) ethanol: 41.87 min and Rt (−)-(*S*)-1-(4′-cyanophenyl)ethanol: 43.04 min. Yield : 29 mg (58%). $[\alpha]_D^{23}$ = −40.9, c = 0.50 MeOH (Mathre et al., 1993, *R* enantiomer $[\alpha]_D^{25} = +41.7$, $c=1.063$, MeOH,). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.50 (d, 3H, CH_3), 2.01 (b, 1H,

Table 1



Entry	Family	Scientific name	Time (days)	Conversion[a]	
				%[b]	e.e.%[c]
1	Alliaceae	*Nothoscordum gracile* (Dryand. ex Aiton) Stearn var. *gracile*	4	42	36 (*S*)
2	Amaranthaceae	*Alternanthera pungens* Kunth.	4	21	79 (*S*)
3	Apiaceae	*Pastinaca sativa* L.	3	91	98 (*S*)
4	Apiaceae	*Conium maculatum* L.	4	43	> 99.9 (*S*)
5	Apiaceae	*Cyclospermum leptophyllum* (Pers.) Sprague var. *leptophyllum*	6	32	91 (*S*)
6	Apiaceae	*Eryngium horridum* Malme	3	96	> 99.9 (*S*)
7	Apocynaceae	*Mandevilla petraea* (A. St.-Hil.) Pichon	3	3	55 (*S*)
8	Berberidaceae	*Berberis ruscifolia* Lam.	5	24	90 (*S*)
9	Bromeliaceae	*Puya spathacea* (Griseb.) Mez.	10	0	nd[d]
10	Cannaceae	*Canna indica* L.	4	7	65 (*S*)
11	Celastraceae	*Maytenus vitis-idaea* Griseb.	4	4	74 (*R*)
12	Euphorbiaceae	*Euphorbia portulacoides* L. *var. portulacoides*	5	20	65 (*S*)
13	Iridaceae	*Iris pseudacorus* L.	7	100	83 (*S*)
14	Nyctaginaceae	*Mirabilis jalapa* L.	4	82	61 (*S*)
15	Orchidaceae	*Cyclopogon elatus* (Sw.) Schltr.	4	4	54 (*S*)
16	Orchidaceae	*Sacoila lanceolata* (Aubl.) Garay	2	5	26 (*S*)
17	Oxalidaceae	*Oxalis articulata* Savigny ssp. *articulata*	4	4	67 (*S*)
18	Plumbaginaceae	*Limonium brasiliense* (Boiss.) Kuntze	10	0	nd[d]
19	Talinaceae	*Talinum paniculatum* (Jacq.) Gaertn.	10	0	nd[d]
20	Talinaceae	*Talinum polygaloides* Gillies ex Arn.	6	22	16 (*R*)

[a] Determined by chiral GC analysis.
[b] Percentage of reduction.
[c] Percentage of enantiomeric excess and absolute configuration between brackets.
[d] nd: not detected.

OH), 4.97 (c, 1H, CH), 7.48–7.50 (d, 2H, ArH), 7.64–7.66 (dd, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=25.46, 69.70, 111.16, 118.86, 126.06, 132.37, 151.05. EI MS *m/z* (rel. int. %)=147 (M^+, 7), 132 (64), 130 (10), 105 (12), 104 (100), 102 (22), 77 (21), 75 (13), 63 (6), 51 (12), 50 (10).

2.6.12. (−)-(S)-1-(4′-Methylphenyl)ethanol:

GC conditions: T_1=100 °C, ΔT=2 °C/min, T_2=200 °C. GC Rt 4′-methylacetophenone: 14.83 min, Rt (+)-(*R*)-1-(4′-methylphenyl) ethanol: 18.13 min and Rt (−)-(*S*)-1-(4′-methylphenyl)ethanol: 19.10 min. Yield: 5 mg (10%). $[\alpha]_D^{23}=-39.7$, *c*=0.051 MeOH (Nakamura and Matsuda, 1998, $[\alpha]_D^{25}=-43.5$, *c*=0.998, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.47 (d, 3H, CH_3), 1.89 (b, 1H, OH), 2.33 (s, 3H, CH_3), 4.86 (c, 1H, CH), 7.15 (d, 2H, ArH), 7.25 (d, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=21.10, 25.09, 70.27, 125.37, 129.18, 137.17, 142.89. EI MS *m/z* (rel. int. %)= 36 (M^+, 39), 121 (98), 119 (9), 117 (5), 93 (100), 92 (21), 91 (77), 77 (45), 65 (27), 51 (15).

2.6.13. (−)-(S)-1-(Thiophen-2-yl)ethanol

GC conditions: T_1=100 °C, ΔT=1 °C/min, T_2=200 °C. GC Rt 2-acetylthiophene: 13.86 min, Rt (+)-(*R*)-1-(thiophen-2-yl)ethanol: 19.33 min and Rt (−)-(*S*)-1-(thiophen-2-yl)ethanol: 20.39 min. Yield: 6 mg (12%). $[\alpha]_D^{23}=-25.1$, *c*=0.054 MeOH (Ohkuma et al., 2000, $[\alpha]_D^{24}=-26.0$, *c*=1.02, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.61 (d, 3 H, CH_3), 1.99 (b, 1 H, OH), 5.14 (c, 1 H, CH), 6.95–6.99 (m, 2 H), 7.23–7.26 (dd, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=25.27, 66.30, 123.20, 124.46, 126.67, 149.87. EI MS *m/z* (rel. int. %) = 128 (M+, 29), 113 (55), 111 (15), 95 (16), 85 (100), 84 (19), 69 (7), 58 (11), 57 (9), 51 (7).

2.6.14. (−)-(S)-1-(Pyridin-2-yl)ethanol

GC conditions: T_1=40 °C (15 min), ΔT=1 °C/min, T_2=160 °C (20 min). GC Rt 2′-acetylpyridine: 37.19 min, Rt (+)-(*R*)-1-(pyridin-2-yl)ethanol: 43,52 min and Rt (−)-(*S*)-1-(pyridin-2-yl)ethanol: 43.94 min. Yield: 24 mg (48%). $[\alpha]_D^{23}=-56.6$, *c*=0.055 MeOH (Ohkuma et al., 2000, $[\alpha]_D^{25}=-58.3$, *c*=0.51 EtOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)=1.51–1.52 (d, 3H, CH_3), 3.64 (b, OH), 4.76 (b, 1H, OH) 4.91 (c, 1H, CH), 7.20–7.23 (m, 1H, ArH), 7.30–7.32 (m, 1H, ArH), 7.69–7.73 (m, 1H, ArH), 8.53–8.54 (d, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)=24.16, 68,87, 119.96, 122.32, 137.08, 147.93, 163.04. EI MS *m/z* (rel. int. %) = 123 (M+, 3), 122 (8), 119 (1), 108 (100), 106 (40), 80 (52), 79 (43), 78 (47), 53 (20), 52 (38), 51 (27).

3. Results and discussion

3.1. Screening of plants

As a part of an evaluation process to find suitable biocatalysts, twenty local weeds were examined using acetophenone as a model substrate. To assure that the roots does not contain the chiral aromatic alcohols or their substrates, a control experiment with roots without adding acetophenone (or any other substrate) were carried out. The crude of these reactions were analyzed by chiral GC and GC–MS, and the presence of neither acetophenones nor the corresponding product of its reduction was observed. The selected plants and the results obtained in this screening are shown in the Table 1.

As can be observed in Table 1, seventeen spices from twenty plants tested produced a reduction of acetophenone to

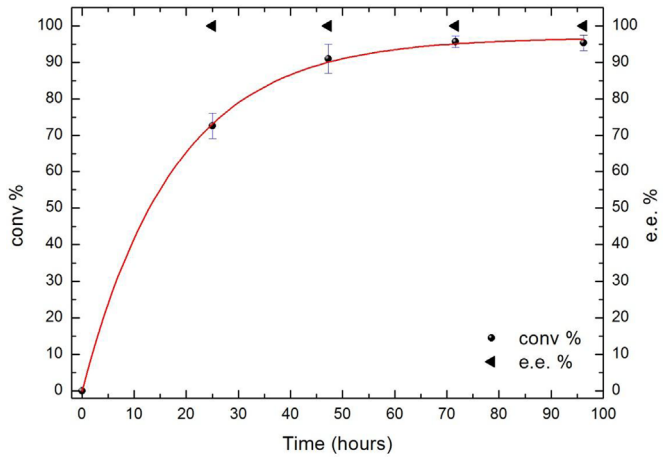


Fig. 1. Kinetic study of the bioreduction of acetophenone mediated by root of *E. horridum*.

1-phenylethanol at differing conversions and enatiomeric excess. According to these data, the *Apiaceae* family produced the best results, with very good enantioselectivity (> 99 −91e.e.%) being obtained with *P. sativa*, *C. maculatum*, *C. leptophyllum* and *E. horridum* (Table 1, entry 3–6). Moreover, it is noteworthy that the best results were obtained using *P. sativa* and *E. horridum* (Table 1, entry 3 and 6), considering also that this represents a new and potential non-traditional use for these plants. This finding is consistent with the fact that *D. carota* (Apiaceae) (Yadav et al., 2002), is the model plant used as the biocatalyst for this type of reaction and moreover, Zilinskas and Sereikaite (2013) recently have reported that *P. sativa* (Apiaceae) was very efficient for the resolution of racemic mixtures of bicyclo[3.3.1]nonane-2,6-dione by stereoselective bioreduction

A quantitative reduction for *I. pseudacorus* with an acceptable enantiomeric excess was obtained (Table 1, entry 13). *M. jalapa* showed good reductive activity but with a low steroselectivity (Table 1, entry 14) whereas *A. punges* revealed good enantioselectivity but a low reductive capacity (Table 1, entry 2). *N. gracile* showed only a moderate reduction of acetophenone and low steroselectivity (Table 1, entry 1). In addition, *B. ruscifolia* this revealed a very good e.e.% (Table 1, entry 8) but had low reductive activity.

Only two of the tested plants had a preference for (*R*)-enantiomer (*anti*-Prelog configuration): *M. vitis-idaea* (Table 1, entry 11) yielded (*R*)-1-phenylethanol in 4% and 74 e.e.% and *T. polygaloides* (Table 1, entry 20) yielded (*R*)-1-phenylethanol in 22% and 16 e.e.%.

P. sphathacea, *L. brasiliense* and *T. paniculatum* did not reveal a capacity for the bioreduction of acetophenone by this methodology (Table 1, entry 9, 18 and 19), with the other plants tested showing a low reductive capacity and variable enantiomeric excess.

It is interesting to note that while *T. paniculatum* showed no reduction of the acetophenone to the corresponding alcohol (Table 1, entry 19), *T. polygaloides* produced a reduction of 22% (Table 1, entry 20).

In order to establish the optimal reaction time a kinetic study using roots of *E. horridum* as the biocatalyst was made, and the results can be observed in (Fig. 1).

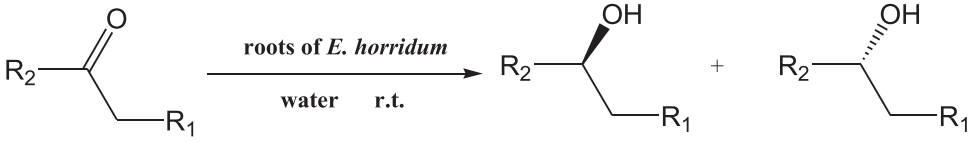
It can be seen in Figure 1 that the reaction reached an optimal performance after 3 days started (96% of conversion), while the enantioselectivity remained very high throughout the study (> 99.9% e.e.), thus revealing the high efficiency and stereoselectivity of the enzymatic complex present in the roots of *E. horridum*.

Additionally, a reaction using fresh root milled with a coffee grinder was made but in this opportunity not conversion of acetophenone was observed. This situation might suggest that cell integrity is necessary and that the reaction occur inside the cells of the root of this plant. However, further studies are needed to support this affirmation.

3.2. Bioreduction of substituted acetophenones

E. horridum is considered to be a problematic weed found in the fields of grazing cattle and it lack of practical use has led to strenuous efforts being made to eradicated (Lallana et al., 2006). It can be easily located and collected free of charge. Now, owing to the results obtained in the reduction of acetophenone with this weed, the possibility to reduce different substituted acetophenones was studied and the results are shown in Table 2.

Table 2



Entry	R_1	R_2	Conversion[a]	e.e.%[a]	Yield %[b]
1	H-	phenyl-	96	> 99.9(*S*)[c]	58
2	H-	2′-chlorophenyl-	100	> 99.9(*S*)	52
3	H-	3′-chlorophenyl-	90	99(*S*)	50
4	H-	4′-chlorophenyl-	94	98(*S*)	56
5	H-	2′-nitrophenyl-	94	> 99.9(*S*)	22
6	H-	3′-nitrophenyl-	95	> 99.9(*S*)	27
7	H-	4′-nitrophenyl-	97	99(*S*)	43
8	H-	4′-fluorophenyl-	90	> 99.9(*S*)	40
9	H-	4′-trifluorophenyl-	100	> 99.9(*S*)	41
10	H-	4′-bromophenyl-	86	99(*S*)	43
11	H-	4′-cyanophenyl-	99	99(*S*)	58
12	H-	4′-methylphenyl-	34	99(*S*)	10
13	H-	2-thyenyl-	42	99(*S*)	12
14	H-	2-pyridyl-	99	> 99.9(*S*)	48

[a] Determined by chiral GC analysis after three days of reaction.
[b] Isolated yield by chromatographic column.
[c] Absolute configuration between brackets.

As can be seen in Table 2, *E. horridum* proved to be a highly esteresoselective biocatalyst (> 98 e.e.%) to obtain different types of chiral benzylic alcohols. It is noteworthy that the conversions were excellent for most of the tested substrates, with the best practical quantitative results achieved using acetophenone, 2'-chloroacetophenone, 4'-trifluoromethylacetophenone, 4'-cyanoacetophenone and 2-acetylpyridine (Table 2, entries 1, 2, 9, 11 and 14, respectively). In contrast, 4'-methylacetophenone and 2-acetylthiophene were only reduced to their corresponding alcohols in moderate to low amounts (Table 2, entries 12 and 13), but still with an equally high stereoselectivity (99 e.e.%). All other substrates tested could be effectively reduced by the biocatalyst, and their products had excellent optical purities. Moreover, it is interesting to note that the biocatalyst reduced all substrates observing Prelog's rule.

A possible influence of the electron-donating nature of the substituent could be observed on the reduction of the carbonyl (Table 2, entries 12 and 13), with 4′-methylacetophenone, and 2-acetylthiophene showing low to moderate conversions to the corresponding alcohols (34% and 42%, respectively). On the other hand, in general, the electron-withdrawing group provided major conversion percentages, and a quantitative conversion was observed when 4′-trifluoromethyloacetophenone and 2′-chlorocetophenone (Table 2, entry 2 and 9) were used as substrates. A similar effect has been observed using *D. carota*, *A. graveolens* and *L. culinaris* as the biocatalysts (Yadav et al., 2002; Liu et al., 2010; Ferreira et al., 2012).

For 2′-, 3′- and 4′-nitroacetophenones (Table 2, entries 5, 6 and 7), an excellent stereo- and chemioselective bioreduction was observed, without reduction of the nitro group. Related to this, it is noteworthy that there are reports in the literature which describe not only the production of alcohols, but also the reduction of the nitro group to amine from nitroacetophenones by using plants as biocatalysts (Ferreira et al., 2012). Moreover, it is interesting to note the importance of these results, since (*S*)-1-(4′-nitrophenyl) ethanol is the pharmacophore found in important drugs such as nifenalol and sotalol (Blay et al., 2010; Pedragosa-Moreau et al., 1997).

It is noteworthy have been reported for *L. culinaris* (Ferreira et al., 2012), and *A. graveolens* (Liu et al., 2010) that steric factors was able to reduce the conversion by the presence of a substituent at the aromatic ring, especially *o*-substituent. Moreover, steric influences produced by substituents on the aromatic ring on the reduction of the carbonyl group were not observed using *E. horridum* as biocatalyst, thus the corresponding nitrophenylalcohols were obtained at very similar conversions.

A similar reactivity to acetophenone was observed with chloroacetophenones, with the conversion to their corresponding alcohols ranging from 90% to 100% (Table 1, entries 2, 3 and 4), but with a somewhat lower enatioselectivity for 4′-chloroacetophenone (98 e.e.%). Additionally, here it should be noted that the importance of these results, since the chlorophenyl ethanols obtained are the pharmacophores in the drugs such as chlorprenaline, solabegron and in the other β_3-adrenergic receptor agonists currently in development (Lu et al., 2011).

While we have not conducted studies to establish the group of enzymes that are responsible for carrying out this transformation, it is probable that the enzyme alcohol dehydrogenase – ketoreductase, dependent on the nicotinamide cofactors as redox equivalents (Blanchard and van de Weghe, 2006; Xie et al., 2009), is involved in the realization of this transformation. However, further studies are needed to support this hypothesis.

It should be noted that the percentages of conversion and enantiomeric excess reported in this paper were higher than those reported by using roots of *D. carota* (Yadav et al., 2002; Baldassarre et al., 2000; Maczka and Mironowicz, 2004; Blanchard and van de Weghe, 2006), *M. esculenta*, and *M. dulcis* (Machado et al., 2006); homogenates of *F. vulgare*, *C. maxima*, *M. sapientum*, and *C. pepo* (Bruni et al., 2006), fruit barks of *P. edulis* (Machado et al., 2008), *Mespilus germanica* and *Citrus reticulata* (Bennamane et al., 2014, 2015). Additionally, it is interesting to note that, unlike the above plants which are used as food, *E. horridum* is a highly invasive weed whose roots have not been previously reported for practical utility. Thus, the detailed results here have identified a potential use for this plant.

The moderate to low yields obtained during purification of the synthesized compounds in same cases was due to the fact that during the extraction procedures it was difficult to break the emulsions formed, which significantly complicated the extraction procedure. Finally, the roots of *E. horridum* remaining after the reaction can be used as manure, thus minimizing chemical waste (Kumaraswamy and Ramesh, 2003).

4. Conclusions

The results present in this paper demonstrate that most of the locally available vegetable species studied have enzyme systems with the necessary ability to reduce prochiral ketones to the corresponding chiral alcohols. As a result of this preliminary study with native undergrowth, it is clear that an unexpected opportunity has arisen to be able to establish new applications for the native flora, especially for those species which do not have any other reported practical utility and are considered to be weeds.

The results obtained here using roots of *E. horridum* as the biocatalyst may offer new strategies for the reduction of selected prochiral phenyl ketones as a critical step in the synthetic organic pathway, thereby avoiding the use of costly and non-renewable metal reducing agents and organic solvents that are commonly utilized in organic synthesis. Moreover, the bioreduction method presented here allows chiral phenyl alcohols to be obtained by using a methodology which is more environmentally friendly than classical reductions of prochiral ketones.

In conclusion, fourteen chiral (*S*) alcohols with very good to excellent enantiomeric excesses were synthesized, thus revealing the roots of *E. horridum* to be a promising biocatalyst for the production of key intermediates. Further investigations are currently ongoing to try to expand the specificity and to explore novel catalytic activities of this new biocatalytic agent.

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