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SHORT COMMUNICATION

Fruits of the glossy privet (*Ligustrum lucidum*—Oleaceae) as biocatalysts for producing chiral aromatic alcohols

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Abstract
The screening of four invasive plant species for use as biocatalysts in the stereoselective reduction of ketones is reported. Our studies revealed that fruits of *Ligustrum lucidum* can be used for the bioreduction of acetophenone to (*S*)-1-phenylethanol (94% conversion, >99 enantiomeric excess [ee]%). Using this methodology, 13 substituted (*S*)-phenylethanols were synthesized with good ee values (>99.9 to 78%) using a technique which is more environmentally friendly than classical reduction of prochiral ketones. The results reveal the fruits of *L. lucidum* to be promising biocatalysts for the production of key intermediates.

Keywords: *Ligustrum lucidum, biocatalysts, bioreduction, substituted acetophenones, chiral aromatic alcohols, green chemistry*

Introduction

The asymmetric reduction of prochiral ketones to produce chiral secondary alcohols is an important reaction in organic chemistry (Singh 1992; Noyori 1994). These alcohols are required as key intermediates in the production of pharmaceuticals and other important chemicals (Yadav 2002 and 2008; Ishihara et al. 2003; Caron et al. 2005).

Despite these reductive processes being of great importance to the chemical industry, comparatively few reduction methodologies have been developed, which take into account the concept of green chemistry (environmentally friendly reaction systems) in order to avoid the formation of toxic waste that may pollute the environment (Wohlgemuth 2010). Compared with conventional chemical processing, biocatalytic asymmetric reductions can offer more selective reactions, environmentally benign processes, and energy-efficient operations. However, due to their low productivity, their commercial application is still limited. Consequently, increasing attention has been paid for developing robust biocatalysts that can carry out reduction processes at a high substrate loading in order to achieve economic feasibility and competitiveness for large-scale biotransformation (Ni & Xu 2012).

During the last decade, chemical reactions using plants as biocatalysts have been the subject of several investigations carried out on the biotransformation of foreign substrates (Cordell et al. 2007). Many transformations of different substrates, such as hydroxylation and oxidation reactions (*Gynostemma pentaphyllum*, Sakamaki et al. 2005), hydrolysis of esters (*Solanum tuberosum* and *Helianthus tuberosus*, Mironowicz 1998), bioreduction of ketones and aldehydes (*Daucus carota, Foeniculum vulgare, Cucurbita pepo, Phaseolus aureus, Cocos nucifera, Saccharum officinarum, Manihot dulcis*, and *M. esculenta*, Cordell

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et al. 2007; Yadav et al. 2002; Bruni et al. 2002; Villa et al. 1998; Kumaraswamy & Ramesh 2003; Fonseca et al. 2009; Baldassarre et al. 2000; Maczka & Mironowicz 2004; Machado et al. 2006; Assunção et al. 2008; Machado et al. 2008; Blanchard & van de Weghe 2006), enzymatic lactonization (*Malus sylvestris* and *H. tuberosus*, Olejniczak et al. 2003), glycosylation (*Ipomoea batatas* and *Eucalyptus perriniana*, Shimoda et al. 2008), etc., have been performed, and have produced very good results. These processes, in general, generate less waste than conventional chemical reagents (Kumaraswamy & Ramesh 2003).

The use of different plant species in the bioreduction of ketones represents an interesting route for the synthesis of useful compounds (Giri et al. 2001; Longo & Sanromaín 2006). The main advantages of using plants as reagents are derived from their easy disposal after use (as they are biodegradable with mild reaction conditions) as well as their wide availability at very low costs. Moreover, the use of functionally intact cells ("whole plant cells") obtained directly from cut portions of plants has emerged, because whole cells can ensure the recycling of the oxidized cofactors (Blanchard & 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).

On the other hand, invasive plants have been considered as one of the most important modern ecological problems, with proliferation affecting conservation of biodiversity (Cohen 2002; Pauchard et al. 2004). One of the most widespread invasive trees in central Argentina is the glossy privet (*Ligustrum lucidum*, W. T. Aiton (Oleaceae)), which is native to China and imported for use as an ornamental plant. Glossy privet has now become a very successful invader of forested sites, resulting in dense stands that eliminate most native vegetation (Hoyos et al. 2010). A similar situation has been observed with *Pyracantha angustifolia* (Franchet) C. K. Schneider (Rosaceae), *Phyracantha atalantioides* (Hance) Stapf (Rosaceae), and *Cotoneaster franchetii* Bois (Rosaceae).

Given these considerations, a project to study the fruits of foreign plants that have become very invasive in the ecosystems of our province was started in order to find some practical use for them. As part of our research into novel biocatalytic systems, the fruits of g*lossy privet*, *yellow firehorn* (*P. angustifolia*), *Gigg's firethorn* (*P. atalantioides*), and *orange cotoneaster* (*C. franchetii*) were selected for studies on the reduction of prochiral phenyl ketones to produce chiral phenyl alcohols.

Methods

General

Ketones and $NaBH_4$ were purchased from Sigma-Aldrich S.A. (Argentina). Sterile deionized water was used to prepare phosphate buffer, and ethyl acetate and hexane were purified by a simple distillation prior to use. The crude reaction products were extracted with ethyl acetate, the organic solutions evaporated, and the products were filtered on a short column with silica gel (70–230 mesh) using hexane–ethyl acetate 90:10 as the eluent. Gas chromatography (GC) analyses were made on a Buck Scientific Model 910/310 instrument with a flame ionization detector (FID) detector, and GC–mass spectrometry (MS) analyses were carried out on a Hewlett Packard HP 5890 Series II gas chromatograph equipped with a Mass Detector HP 5970. The ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 MHz, and optical rotations were measured in a JASCO P-1010 polarimeter. All products were identified by comparing their MS, and ^{1}H and ^{13}C NMR spectra with literature data.

GC-FID and GC–MS analyses

To establish the chromatographic conditions, all substrates used were reduced with sodium borohydride in ethanol to obtain a racemic mixture of the corresponding alcohols with good yields. The GC separations were performed on a fused silica capillary column Supelco β-Dex120 (phenyl polysiloxane with 20% of permethylated β-cyclodextrin, 30 m, 0.25 mm, 0.25 μm) with GC general conditions of split, 145 mL/min; injector, 220°C; detector FID, 220°C; carrier gas, N_2; and head pressure, 29 psi with the conversion percentages of the reactions being determined by GC using normalized peak areas without a correction factor. The GC–MS (70 eV) analyses were performed using the same conditions as those in the GC analysis, but using a Hewlett Packard HP-5 (Crosslinked 5% PhMe Siloxane, 30 m, 0.32 mm, 0.25 μm film thickness) capillary column.

Biocatalysts

Healthy and mature fruits of *L. lucidum*, *P. angustifolia*, *P. atalantioides*, and *C. franchetii* were collected between April and August in Córdoba City (Argentina), and identified by a botanist. To carry out this study, the fruits were washed with tap water to remove traces of dirt and then disinfected by immersion in a 5% sodium hypochlorite solution for 15–20

min. These were then washed again, but with sterile deionized water. The fruits were used immediately after collection to assure the integrity of the enzymatic system.

Bioreductions

These reactions were conducted immediately after acquisition of the fruits. Using a coffee bean grinder that had been previously disinfected, the fruits were ground to form a slurry. This slurry (20 g) was added to a sterile Erlenmeyer flask (250 mL) with phosphate buffer (150 mL, pH: 6.5), and then the ketone (100 mg) was dissolved in dimethyl sulfoxide (DMSO) (1.25% v/v) and added to this suspension. The reaction was carried out by stirring on an orbital shaker (120 rpm) for six days at 20°C, with the flask being closed. Finally, the crude reaction was filtered through cotton in order to remove particles in suspension, and the solution was extracted with 40 mL of ethyl acetate, with very gentle stirring to avoid formation of an emulsion. 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.

Kinetic study of the bioreduction of acetophenone

In order to establish the optimal reaction time, a kinetic study was made using acetophenone as the model substrate. The reaction progress was monitored by taking samples (5 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 (1 μL) by GC using the same general conditions described in *GC-FID and GC–MS analyses*. The reactions were made in triplicate.

Scale reduction

Ground ripe fruits of *L. lucidum*, 132 g and phosphate buffer, pH: 6.5 (1000 mL) were placed into a conical flask. Acetophenone (0.670 g, 5.5 mmoles) dissolved in 12.5 ml of DMSO was added to the slurry of *L. lucidum* in the above buffer, covered and allowed to shake for 6 days at 20°C. Then the slurry of glossy privet was filtered off and washed with deionized water (4 × 250 ml). The combined aqueous filtrate was extracted with ethyl acetate (500 ml). The organic layer was dried and the crude product was obtained (0.802 g). Pure (*S*)-1-phenylethanol was recovered after column chromatography with silica gel using hexane/ethyl acetate 95/5 as eluent; conversion (by GC analysis using the same conditions described in *GC-FID and GC–MS analyses*): 75%; ee, > 99%; yield, 241 mg (36%).

GC and spectroscopic data

All products were identified by comparing their GC retention times, MS, and ^{1}H and ^{13}C NMR spectra with literature data (Salvi & Chattopadhyay 2001 and 2008; Yu et al. 2011; Cheemala et al. 2007; Matsugi et al. 2004).

(−)-(*S*)-*1-Phenylethanol*: GC conditions: $T_1 = 80°C$ (1 min), $\Delta T = 2.5°C$/min, $T_2 = 140°C$. GC Rt acetophenone: 15.15 min, Rt (+)-(*R*)-1-phenylethanol: 20.88 min, and Rt (−)-(*S*)-1-phenylethanol: 21.58 min. $[\alpha]^{23}_{D} = -44.7$, c = 0.51 MeOH (Yadav et al. 2002, $[\alpha]^{25}_{D} = -39.1$, $c = 3.5$ 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. Electron ionization (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).

(−)-(*S*)-*1-(2′-Chlorophenyl)ethanol*: GC conditions: $T_1 = 110°C$, $\Delta T = 3°C$/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. $[\alpha]^{23}_{D} = -60.3$, c = 0.5 MeOH (Nakamura & Matsuda 1998, $[\alpha]^{25}_{D} = -62.7$, $c = 0{,}894$ $CHCl_3$). ^{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).

(−)-(*S*)-*1-(3′-Chlorophenyl)ethanol*: GC conditions: $T_1 = 110°C$ (1 min), $\Delta T = 3°C$/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. $[\alpha]^{23}_{D} = -41.1$, c = 0.48 MeOH (Nakamura & Matsuda 1998, $[\alpha]^{25}_{D} = -43.5$, $c = 1.08$, $CHCl_3$). ^{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).

(−)-(*S*)-*1-(4′-Chlorophenyl)ethanol*: GC conditions: $T_1 = 110°C$, $\Delta T = 3°C/min$, $T_2 = 210°C$ (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. $[\alpha]^{23}_D = -45.9$, $c = 0.38$ MeOH (Nakamura & Matsuda 1998, $[\alpha]^{25}_D = -49.0$, $c = 1.84$, 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).

(+)-(*S*)-*1-(2′-Nitrophenyl)ethanol*: GC conditions: $T_1 = 100°C$, $\Delta T = 2°C/min$, $T_2 = 210°C$ (20 min). GC Rt 2′-nitroacetophenone: 31.15 min, Rt (−)-(*R*)-1-(2′-nitrophenyl)ethanol: 40.48 min, and Rt (−)-(*S*)-1-(2′-nitrophenyl)ethanol: 42.23 min. $[\alpha]^{23}_D = +18.0$, $c = 0.022$ MeOH (Comasseto et al. 2006, $[\alpha]^{25}_D = +30.6$, $c = 3.06$ $CHCl_3$). ^{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).

(−)-(*S*)-*1-(3′-Nitrophenyl)ethanol*: GC conditions: $T_1 = 100°C$, $\Delta T = 2°C/min$, $T_2 = 210°C$ (20 min). GC Rt 3′-nitroacetophenone: 34.18 min, Rt (+)-(*R*)-1-(3′-nitrophenyl)ethanol: 45.97 min, and Rt (−)-(*S*)-1-(3′-nitrophenyl)ethanol: 46.35 min. $[\alpha]^{23}_D = -27.9$, c = 0.44 MeOH (Comasseto et al. 2006, $[\alpha]^{25}_D = -30.5$, $c = 2.99$ $CHCl_3$). ^{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).

(−)-(*S*)-*1-(4′-Nitrophenyl)ethanol*: GC conditions: $T_1 = 100°C$, $\Delta T = 2°C/min$, $T_2 = 210°C$ (20 min). GC Rt 4′-nitroacetophenone: 37.18 min, Rt (+)-(*R*)-1-(4′-nitrophenyl)ethanol: 48.66 min, and Rt (−)-(*S*)-1-(4′-nitrophenyl)ethanol: 49.96 min. $[\alpha]^{23}_D = -30.0$, c = 0.55 MeOH (Yadav et al. 2002, $[\alpha]^{25}_D = -30.5$, $c = 4$ $CHCl_3$). ^{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).

(−)-(*S*)-*1-(4′-Fluorophenyl)ethanol*: GC conditions: $T_1 = 100°C$, $\Delta T = 2°C/min$, $T_2 = 200°C$. 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. $[\alpha]^{23}_D = -35.2$, c = 0.045 (Nakamura & Matsuda 1998, $[\alpha]^{25}_D = -37.7$, $c = 0.931$, 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).

(−)-(*S*)-*1-(4′-Bromophenyl)ethanol*: GC conditions: $T_1 = 100°C$, $\Delta T = 2°C/min$, $T_2 = 200°C$ (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. $[\alpha]^{23}_D = -35.1$, c = 0.46 MeOH (Nakamura & Matsuda 1998, $[\alpha]^{25}_D = -37.9$, $c = 1.13$, $CHCl_3$). ^{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).

(−)-(*S*)-*1-(4′-Trifluorophenyl)ethanol*: GC conditions: $T_1 = 100°C$, $\Delta T = 2°C/min$, $T_2 = 200°C$. 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. $[\alpha]^{23}_D = -26.3$, c = 0.066 MeOH (Nakamura & Matsuda 1998, $[\alpha]^{25}_D = -28.1$, $c = 1.13$, 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).

(−)-(*S*)-*1-(4′-Cyanophenyl)ethanol*: GC conditions: $T_1 = 100°C$, $\Delta T = 2°C/min$, $T_2 = 200°C$. 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. $[\alpha]^{23}_D = -40.0$, c = 0.44 MeOH (Mathre et al. 1993,

R enantiomer $[\alpha]^{25}_D = +41.7$, $c = 1.063$, MeOH,). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 1.50 (d, 3H, CH_3), 2.01 (b, 1H, 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).

(−)-(*S*)-*1-(4′-Methylphenyl)ethanol*: GC conditions: $T_1 = 100$°C, $\Delta T = 2$°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. $[\alpha]^{23}_D = -40.4$, c = 0.049 MeOH (Nakamura & Matsuda 1998, $[\alpha]^{25}_D = -43.5$, $c = 0.998$, 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. %) = 136 (M^+, 39), 121 (98), 119 (9), 117 (5), 93 (100), 92 (21), 91 (77), 77 (45), 65 (27), 51 (15).

(−)-(*S*)-*1-(Thiophen-2-yl)ethanol*: GC conditions: $T_1 = 100$°C, $\Delta T = 1$°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. $[\alpha]^{23}_D = -22.3$, c = 0.051 MeOH (Ohkuma et al. 2000, $[\alpha]^{24}_D = -26.0$, $c = 1.02$, $CHCl_3$). ^{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).

(−)-(*S*)-*1-(Pyridin-2-yl)ethanol*: GC conditions: $T_1 = 40$°C (15 min), $\Delta T = 1$°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. $[\alpha]^{23}_D = -53.9$, c = 0.056 MeOH (Ohkuma et al. 2000, $[\alpha]^{25}_D = -58.3$, $c = 0.51$ 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).

Results and discussion

Reactions using acetophenone as a model substrate

In our attempt to produce chiral aromatic alcohols in high ee, acetophenone **1** was used as a test substrate and it was found that ground fresh fruits of *L. lucidum* could be used for enantioselective bioreduction of **1** to give (−)-(*S*)-1-phenylethanol **2** in 94% of conversion with an excellent enantioselectivity (ee% > 99, Scheme 1).

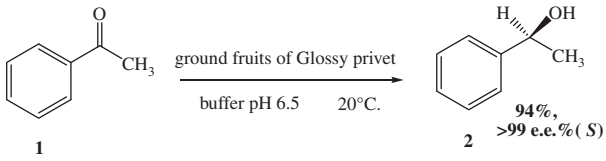
To assure that the fruit did not contain the chiral aromatic alcohols or their substrates, a control experiment with fruits alone, without adding acetophenone (or any other substrate) was carried out. The crude of this reaction was analyzed, and neither the presence of acetophenone nor the corresponding product of its reduction was observed.

In order to establish the optimal reaction time, a kinetic study using ground ripe fruits of *L. lucidum* as the biocatalyst was made (Figure 1).

It can be seen in Figure 1 that the reaction reached optimal performance after 6 days (94% of conversion), while the enantioselectivity remained very high throughout the study (> 99% ee), revealing the high efficiency and stereoselectivity of the enzymatic complex present in the fruits of *L. lucidum*. 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 (Xie et al. 2009; Blanchard & van de Weghe 2006)—is involved in this transformation. However, further studies are needed to support this hypothesis.

To demonstrate the viability of this bioreduction protocol, a major scale reduction of acetophenone (≈ 0.7 g batch) was conducted. Although, the extent of chemical conversion of **1** decreased (94% to 75%), the product **2** was obtained with an excellent ee% (> 99%).

A reaction using unripe fruits of *L. lucidum* was also performed and the outcome was similar, but with slightly lower values (84% conversion, 98% ee) than those achieved using ripe fruits. Moreover, other reactions using ripe fruits of *L. lucidum* were performed using water as solvent. In this case the



Scheme 1. Conversion of acetophenone to (-)-(*S*)-1-phenylethanol using fruits of *L. lucidum* as the biocatalyst.

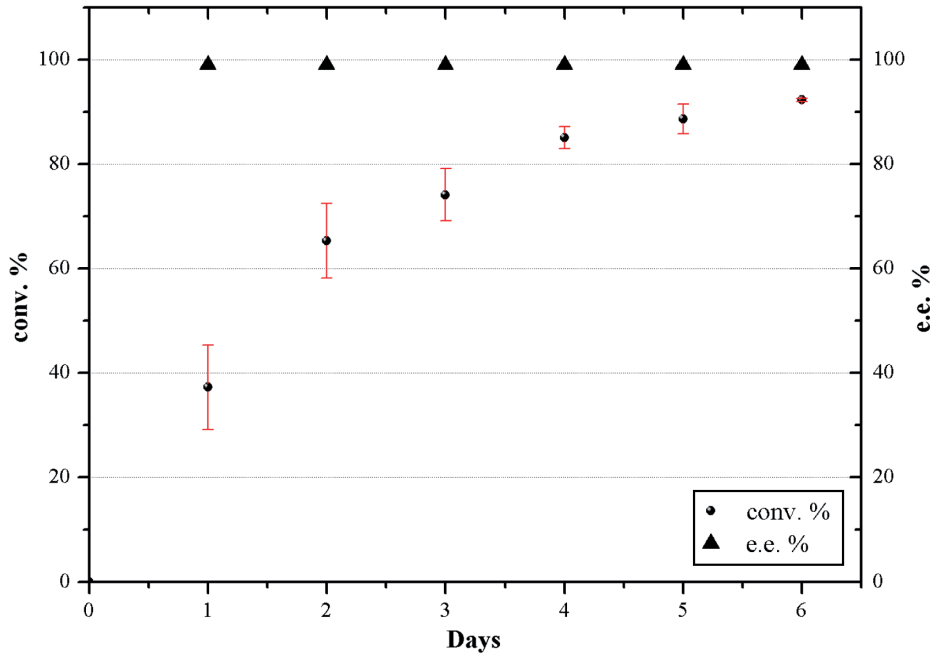


Figure 1. Kinetic study of the bioreduction of acetophenone catalyzed by *L. lucidum*.

outcome was similar, but with slightly lower values (82% conversion, 95% ee) than those achieved using a solution of phosphate buffer at pH: 6.5 as solvent.

One of the major disadvantages of using fruits of plants as sources of enzymes capable of producing chemical reactions is that plants only produce fruits for limited and specific seasons of the year. To address this situation, fruits of *L. lucidum* were frozen and kept for 1–2 weeks at − 20° C. Then, the fruits were ground and a reaction was performed using acetophenone as the substrate. However, in this case it was observed that the reductive capacity of the frozen fruit decreased significantly (9% conversion, 81% ee).

It should be noted that, although there are several reports in the literature on the use of plant parts as biocatalysts in the reduction of prochiral ketones, few studies have been conducted using fruits. In the results reported in this communication, conversion (%) and ee(%) achieved in the reduction of acetophenone are better than those described using *M. pumila* (40.9%, 81.5 ee%(*R*), Yang et al. 2008), *C. nucifera* (79%, 95 ee%(*S*), Fonseca et al. 2009), *Passiflora edulis* (74%, 23 ee%(*S*), Machado et al. 2008), and *Lycopersicum esculentum* (80%, 95 e.e%(*S*), Phukan & Devi 2012). Moreover, it is interesting to note that, unlike the above fruits, *L. lucidum* is a highly invasive weed whose fruit has not been previously reported for practical use. Thus, the results detailed here have identified a potential practical use for the fruits of this plant.

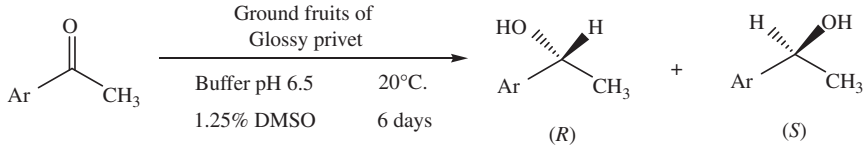
Also, other potential tree biocatalysts also were screened under the same conditions for the above reduction. The ground fresh fruits of *P. angustifolia*, *P. atalantioides*, and *C. franchetii* were also tested and they yielded 59%, 56%, and >99 ee%, respectively, but with a conversion to alcohol **2** of 76%, 26%, and 5%, respectively, after 6 days of reaction.

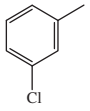
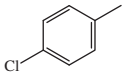
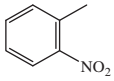
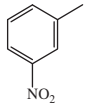
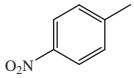
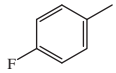
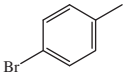
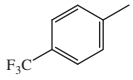
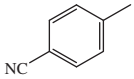
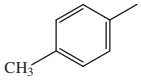
Bioreduction of substituted prochiral phenyl ketones by L. lucidum

The results observed using *L. lucidum* as biocatalyst in the acetophenone reductions encouraged us to study the reduction of different substituted acetophenones in the same system (Table I). This showed (Table I) that fruits of glossy privet were efficient bioreducers of a number of prochiral aromatic ketones to their corresponding alcohols, with conversions ranging from 79 to 98%, except for 2′-nitroacetophenone, 4′-methylacetophenone, and 2-acethylthiophene (conversions from 7 to 48%; Table I, entries 5, 12, and 13). All other substrates tested could be effectively reduced by the biocatalyst, and most of their products had satisfactory optical purities. It is interesting to note that the biocatalyst reduced all substrates observing Prelog's rule.

An influence of electron-donating substituents on the reduction of the carbonyl group was evident, producing the corresponding alcohol at moderate-to-low conversions (Table I, entries 12 and 13). Thus, 4′-methylacetophenone showed moderate conversion (48%) and 2-acetylthiophene showed low

Table I. Ability of *L. lucidum* to reduce different substituted prochiral ketones.



Entry	Ar	Conversion %[a]	ee%[b]	Isolated yield %[c]
1		94	>99 (*S*)	79
2		90	>99.9 (*S*)	20
3		93	97 (*S*)	26
4		92	91 (*S*)	38
5		7	88 (*S*)	traces
6		79	83 (*S*)	45
7		98	87 (*S*)	49
8		90	97 (*S*)	57
9		82	81 (*S*)	13
10		84	78 (*S*)	5
11		97	95 (*S*)	30
12		48	88 (*S*)	14
13		17	79 (*S*)	7
14		82	81 (*S*)	52

[a]Measured by GC analysis.
[b]ee values were determined by chiral GC analysis. Absolute configurations were assigned by comparing the retention time of GC data with the reports in the literature and by comparison of the sign of optical rotations with the literature values.
[c]Isolated by column chromatography.

conversion to the corresponding alcohol (17%). In contrast, electron-withdrawing groups increased the rate of conversion, with practically quantitative conversion being observed with 4′-cyanoacetophenone (97% conversion, Table I, entry 11) and 4′-nitroacetophenone (98% of conversion, Table I, entry 17) as substrates. A similar situation has been observed using *D. carota*, *Apium graveolens*, and *Lens*

culinaris as the biocatalysts (Yadav et al. 2002; Liu et al. 2010; Ferreira et al. 2012).

For 2′-, 3′-, and 4′-nitroacetophenones (Table I, entries 5, 6, and 7), very good stereo- and chemoselective bioreduction was also observed, without reduction of the nitro group. There are reports in the literature which describe not only the production of alcohols, but also the reduction of the nitro group to amines from nitroacetophenones using plants as biocatalysts (Ferreira et al. 2012). However, in this study, nitroacetophenones showed a very variable percentage of conversion, and in the case of 2′-nitroacetophenone there was possible steric influence on the reduction of the carbonyl group, giving very low conversion rates (7%, Table I, entry 5). A similar situation was observed by Ferreira et al. (2012) in the reduction of 2′-nitroacetophenone using *L. culinaris* as the biocatalyst. On the other hand, Liu *et al.* (2010) using *A. graveolens* as biocatalyst showed that the presence of a substituent on the aromatic ring, especially an o-substituent, caused steric hindrance, reducing the rate of reaction. It is noteworthy that (S)-1-(4′-nitrophenyl) ethanol, which can be produced in this reaction, is the pharmacophore found in important drugs such as nifenalol and sotalol (Pedragosa-Moreau et al. 1997; Blay et al. 2010).

The ee observed for all reactions ranged from very good (4′-trifluoromethylacetophenone 78%; Table I, entry 10) to excellent (2′-chloroacetophenone $>99.9\%$; Table I, entry 2). A reactivity similar to that of acetophenone was observed with chloroacetophenones, with the conversion to their corresponding alcohols ranging from 90 to 93%, but with the enantioselectivity decreasing in the sequence 2′-, 3′-, and 4′-chloroacetophenone (>99.9, 97, and 91 ee%, respectively; Table I, entries 2, 3, and 4). It is interesting to note the importance of these results, since the three chlorophenyl ethanols obtained are pharmacophores in drugs such as chlorprenaline, solabegron, and in the other β_3-adrenergic receptor agonists currently in development (Lu et al. 2011).

The moderate-to-low yields obtained during purification of the synthesized compounds was due to the fact that during the extraction procedures, it was difficult to break the emulsions formed, which significantly complicated the extraction procedure.

Currently, we are conducting studies to find a good technique for preserving the activity of the fruits of glossy privet, and thereby eliminate the dependence on their seasonal availability. A green and biodegradable biocatalyst, such as this, can be used as manure after service in the synthetic reaction, thus minimizing the waste of chemicals (Kumaraswamy & Ramesh 2003).

Conclusions

In conclusion, several chiral (*S*) alcohols with excellent or very good enantiomeric excesses (>99.9-78%) were synthesized, thus revealing the fruits of *Ligustrum lucidum* to be a promising biocatalyst for the production of key intermediates. In addition, the presence of substituents (electron-withdrawing or electron-donating) was observed in the aromatic ring, which could have steric and/or electronic influences on the course of reduction.

The results obtained here using fruits of glossy privet 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. As a result of this study with wild foreign plants, it is clear that an unexpected opportunity has arisen to establish new applications for the foreign flora, especially for those species which do not have any other reported practical utility and are considered to be highly invasive weeds. The bioreduction method presented here allows chiral phenyl alcohols to be obtained with a very good enantioselectivity by using a methodology which is more environmentally friendly than classical reductions of prochiral ketones.

Further investigations are currently ongoing, trying to expand the specificity and exploring new catalytic activities of this new inexpensive biocatalytic agent.

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