# Graphical Abstract

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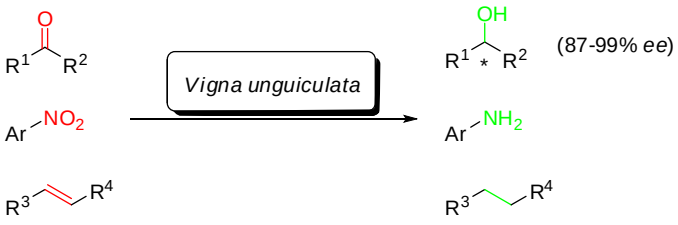

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# Reduction processes biocatalyzed by *Vigna unguiculata*

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**Abstract**—Whole cells from the Brazilian beans *feijão da corda* (*Vigna unguiculata*) have been employed as biocatalyst in different bioreduction processes. Good to excellent selectivities can be obtained in the reduction of aromatic and aliphatic ketones, as well as β-ketoesters, depending the conversions and the chemoselectivity on the substrate structure. This biocatalyst was also able to reduce the nitro moiety of different aromatic nitro compounds, presenting also enoate reductase activity, chemoselectively catalyzing the double bond reduction of 4-phenyl-3-buten-2-one with moderate conversion. 

## 1. Introduction

Nowadays the preparation of enantioenriched secondary alcohols presents an increasing interest due to their numerous applications as chiral building blocks in pharmaceutical or (agro)chemical industry and their activity as pheromones and flavor or aroma enhancing compounds.[1] Different methodologies have been developed in order to synthesize these important compounds,[2] being the stereoselective reduction of ketones one of the most widely employed methods, since it allows the obtaining of these derivatives in quantitative yields.[3] In the last few years, biocatalysis has emerged as a reliable alternative to conventional chemical methods due to the high selectivities achieved while employing mild, economically viable and eco-compatible conditions.[4] Optically active *sec*-alcohols can be obtained employing a variety of biocatalytic methods[5] involving, *i.e.* lipase-catalyzed kinetic resolutions, but in the last few years the synthesis of enantiopure secondary alcohols through reduction of ketones or oxidative kinetic resolutions of racemic alcohols using alcohol dehydrogenases (ADHs) has gained increasing interest.[6]

On the other hand, the selective reduction of aromatic nitro compounds is a useful transformation applied to the synthesis of amines, important intermediates in the production of pharmaceuticals and other high added value derivatives.[1b,7] This reaction also presents interest in bioremediation processes. For example, nitrophenols are widely used as industrial internediate compounds, but they are environmental pollutants due to their toxicity and resistance to biodegradation. Several methodologies have been described to perform the chemical reduction of nitro derivatives.[8] Enzymatically, examples of this process have been reported employing nitroreductases whereas other authors have described the use of flavoenzymes for the hydrogenation of dinitro compounds.[9]

Plants have been considered as enzymatic systems suitable for being employed in biocatalysis. Most of the reactions catalyzed by plants are redox processes (reduction of aldehydes and ketones, oxidation of racemic alcohols), but they are also able to catalyze hydrolyses of esters and acylations of alcohols.[10] Immobilized plant cell cultures as well as whole cell tissues present the advantages of wide availability at low costs, mild reaction conditions and easy removal after use.

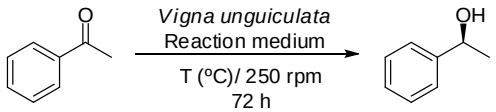
In some previous studies performed by our research groups, several biocatalytic systems obtained from the Brazilian biodiversity have been analyzed and employed in the reduction of prochiral ketones and the acylation of racemic alcohols.[11] Herein, we describe the use of whole cells of *Vigna unguiculata* as biocatalyst in different organic synthetic reactions. The so-called *feijão de corda* or *feijão caupi* belongs to the *Phaseoleae* family and grows predominantly in the North and Northeast of Brazil, due to its resistance to high temperatures and water stress. This legume presents high interest in the Brazilian diet, since it is an important source of proteins (23-25% on average) and

carbohydrates with high contents of fiber, vitamins and minerals. In the present manuscript we will show that this preparation is able to reduce carbonyl, nitro and alkene compounds, demonstrating its potential as catalyst in organic synthesis.

## 2. Results and discussion

Our initial efforts were devoted to employ cells of *Vigna unguiculata* as biocatalyst in the reduction of a group of aromatic ketones. Acetophenone (**1a**) was chosen as model substrate for the optimization of the reaction conditions. A first set of bioreductions were performed at 30ºC during 72 h in order to determine the best reaction medium for this process as shown in Table 1 (entries 1-4). When the bioreduction was performed in water, a 34% of the Prelog alcohol (*S*)-**1b** was obtained with 92% *ee* (entry 1). The use of a Tris-HCl buffer at pH 7.5 led to a slight decrease in the enzymatic activity (entry 2). Then, we decided to test some cosolvents when water was employed. The addition of 2% v $v^{-1}$ $^{i}$PrOH allowed the obtaining of enantiopure (*S*)-**1b** with a 35% conversion (entry 3). When DMSO was employed in the same amount (entry 4), the optical purity of the resulting alcohol was lower, while no appreciable change was observed in the biocatalyst activity.

**Table 1**. *Vigna unguiculata* biocatalyzed reduction of **1a** to (*S*)-**1b** in aqueous media at different reaction conditions.[a]




**1a** (*S*)-**1b**

| Entry | % Cosolvent | T (ºC) | *c* (%)[b] | *ee* (%)[b] |
|---|---|---|---|---|
| 1 | None | 30 | 34 | 92 |
| 2[c] | None | 30 | 31 | 92 |
| 3 | 2% $^{i}$PrOH | 30 | 35 | ≥99 |
| 4 | 2% DMSO | 30 | 33 | 96 |
| 5 | 5% $^{i}$PrOH | 30 | 32 | ≥99 |
| 6 | 10% $^{i}$PrOH | 30 | 24 | ≥99 |
| 7 | 30% $^{i}$PrOH | 30 | ≤3 | n.d. |
| 8 | 2% $^{i}$PrOH | 20 | 35 | ≥99 |
| 9 | 2% $^{i}$PrOH | 37 | 28 | ≥99 |
| 10 | 2% $^{i}$PrOH | 45 | 19 | 98 |

[a] Reaction time, 72 h. For other reaction conditions see the Experimental Section.

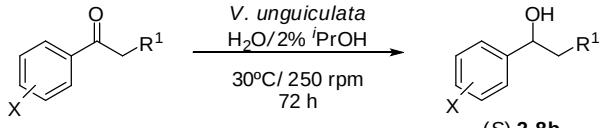
[b] Conversions (*c*) and enantiomeric excesses (*ee*) were determined by HPLC or GC.

[c] Reaction performed using a Tris-HCl 50 mM buffer (pH 7.5).

n.d. not determined.

Once water containing $^{i}$PrOH was selected as the best reaction medium for the biotransformations, we analyzed the effect of this cosolvent on the biocatalytic properties of *V. unguiculata*. Bioreductions of **1a** were carried out employing different concentrations of $^{i}$PrOH, as shown in entries 5-7. The use of 5% v $v^{-1}$ $^{i}$PrOH did not alter the properties of the enzyme, obtaining enantiopure (*S*)-**1b** with 32% conversion (entry 5). Higher cosolvent concentrations led to a progressive deactivation of the biocatalyst, as indicated in entries 6 and 7. This effect was especially dramatic when adding 30% v $v^{-1}$ $^{i}$PrOH, since no reduction was observed in these conditions. Temperature was also analyzed as a key parameter in the acetophenone bioreduction (entries 8-10). When it was carried out at 20ºC, the same activity and selectivity was achieved than working at 30ºC (entry 8). The increase of the temperature to 37ºC (entry 9), led to a slight deactivation of the biocatalyst, while at 45ºC (entry 10) the loss of the activity was higher (*c*=19%), not observing significant changes in the optical purity of (*S*)-**1b**.

**Table 2**. Bioreductions of substituted acetophenones catalyzed by whole cells of *Vigna unguiculata* (t=72 h).[a]




**2-12a** (*S*)-**2**-**8b** (*R*)-**9**-**12b**

| Entry | Ketone | X | $R^1$ | (%) **2-12b**[b] | *ee* (%)[b] |
|---|---|---|---|---|---|
| 1 | **2a** | 4'-MeO | H | 5 | ≥99 (*S*) |
| 2 | **3a** | 3'-MeO | H | 86 | ≥99 (*S*) |
| 3 | **4a** | 2'-MeO | H | 5 | ≥99 (*S*) |
| 4 | **5a** | 4'-Me | H | 13 | 96 (*S*) |
| 5 | **6a** | 2'-Me | H | 11 | ≥99 (*S*) |
| 6 | **7a** | 4'-$^{i}$Pr | H | 13 | 87 (*S*) |
| 7 | **8a** | 4'-Cl | H | 24 | ≥99 (*S*) |
| 8 | **9a** | 3'-Br | H | 56 | ≥99 (*R*) |
| 9 | **10a**[c] | 4'-Cl | Cl | 69 | ≥99 (*R*) |
| 10 | **11a**[c] | 2',4'-Cl | Cl | 78 | ≥99 (*R*) |
| 11 | **12a**[c] | 3',4'-Cl | Cl | 78 | 89 (*R*) |

[a] For more detailed reaction conditions, see the Experimental Section.

[b] Conversions (*c*) and enantiomeric excesses (*ee*) were determined by HPLC or GC. Absolute configuration of the alcohol is in brackets.

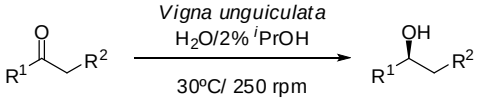
[c] Absolute configuration is reversed due to a change in the substituent priority according to the CIP sequence rules.

After establishing the best reaction conditions for the bioreduction of **1a**, we extended our study to other acetophenone derivatives, as indicated in Table 2. For most of the ketones studied, the expected Prelog product was formed, with the exception of substrate **9a** (3'-bromo), where the anti-Prelog alcohol (*R* configuration) was obtained, indicating that probably for this compound different alcohol dehydrogenase(s) were acting in the enzymatic system. Firstly, we analyzed the effect of an electron-donating group such as methoxy, observing that the position of this moiety greatly affected the enzymatic activity. While the 4'- and 2'-methoxy derivatives (**2a** and **4a**, respectively), showed very low conversions after 72 h (entries 1 and 3), (*S*)-**3b** could be obtained in an 86% extent (entry 2). In all these processes, alcohols were isolated in

enantiopure form. Substituted acetophenones with an alkyl group in the aromatic ring were also reduced although in a lower extent. While ketones **5a** and **6a** were reduced with conversions close to 13% to the corresponding enantiopure *S*-alcohols (entries 4 and 5), 4'-isopropylacetophenone **7a** led to (*S*)-**7b** with the same conversion but lower optical purity (*ee*=87%), indicating that the presence of bulkier alkyl chains had a negative effect in the biocatalyst selectivity (entry 6). Halogenated ketones were also tested obtaining better results. Thus, 4'-chloroacetophenone was converted to (*S*)-**8b** with excellent enantiomeric excess and 24% conversion after 72 h (entry 7). The bromo derivative **9a** (entry 8) led to the opposite enantiopure (*R*)-**9b** with good conversion (*c*=56%).

A set of α-chloro ketones was also tested as possible substrates for the enzymatic processes. Reduction of these compounds lead to the formation of the corresponding halohydrins, versatile intermediates in the preparation of bioactive compounds.[12] When 2-chloroacetophenone **10a** was reduced in the presence of *V. unguiculata* (entry 9), the Prelog alcohol (*R*)-**10b** was obtained with good yield and excellent selectivity. The 2,2',4'-trichloro derivative **11a** (entry 10) also led to enantiopure (*R*)-**11b** with high conversion (*c*=78%), while bioreduction of ketone **12a** (entry 11) occurred with the same yield, but with a lower selectivity [(*R*)-**12b** was obtained with *ee*=89%].

**Table 3**. Bioreduction of aliphatic ketones and β-ketoesters employing *Vigna unguiculata* as biocatalyst.[a]




**13**-**16a** (*S*)-**13**-**15b** (*R*)-**16b**

**13a** $R_1$=-$CH_2(CH_2)_4CH_3$ $R_2$=H
**14a** $R_1$=-$CH_2(CH_2)_7CH_3$ $R_2$=H
**15a** $R_1$=-$CH_2COOCH_3$ $R_2$=H
**16a** $R_1$=-$CH_2COOCH_3$ $R_2$=Cl

| Entry | Substrate | t (h) | *c* (%)[b] | *ee* (%)[b] |
|---|---|---|---|---|
| 1 | **13a** | 72 | 16 | ≥99 (*S*) |
| 2 | **14a** | 72 | 19 | ≥99 (*S*) |
| 3 | **15a** | 48 | 83 | ≥99 (*S*) |
| 4[c] | **16a** | 48 | 88 | ≥99 (*R*) |

[a] For reaction details, see the Experimental Section.

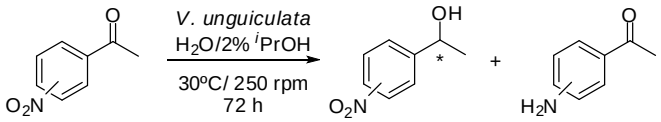
[b] Conversion (*c*) and enantiomeric excesses (*ee*) were determined by HPLC or GC. Absolute configuration of the alcohol is in brackets.

[c] Absolute configuration is reversed due to a change in the substituent priority according to the CIP sequence rules.

Aliphatic ketones and β-ketoesters were also tested as suitable substrates for *V. unguiculata* (Table 3). Bioreduction of 2-octanone (**13a**) led to the formation of enantiopure (*S*)-**13b** with low conversion (*c*=16%) after 72 h (entry 1). Enantiopure (*S*)-2-undecanol (**14b**) could be obtained with a slightly higher conversion at the same reaction time, as shown in entry 2. Whole cells were tested in the reduction of β-ketoesters which led to the corresponding chiral β-hydroxyesters, important synthons in organic chemistry.[13] Ethyl acetoacetate (**15a**) was reduced with high conversion (*c*=83% after 48 hours, entry 3) leading to enantiopure (*S*)-**15b**, indicating that *V. unguiculata* showed a high activity for this compound (complete conversion was achieved after 72 h). A similar result was obtained in the bioreduction of **16a** (entry 4). After 48 h, 88% of (*R*)-**16b** with 99% *ee* was obtained. This alcohol is a useful chiral building block for the synthesis of different pharmaceuticals.[14]

*V. unguiculata* cells were tested in the reduction of acetophenones presenting in their structure a nitro substituent in the aromatic ring, group also sensitive in reduction processes, in order to analyze the chemoselectivity of these reactions (Table 4). Thus, bioreduction of 4'-nitroacetophenone (**17a**) led after 72 h to the formation of two different products: the expected enantiopure nitro alcohol (*R*)-**17b** with 42% conversion, but also the amino ketone obtained from the reduction of the nitro group (**17c**), was achieved in a 54% extent (entry 1). Thus, this biocatalytic system presented enzyme(s) able to reduce the nitro moiety into the corresponding amine. Bioreduction of 3'-nitroacetophenone (**18a**) occurred with low conversion compared to the 4'-nitro derivative (entry 2). Only 14% of enantiopure (*S*)-**18b** was achieved, while 3'-aminoacetophenone (**18c**) was formed in an 8% extent. Lower activity was measured for the 2'-nitro derivative **19a** (entry 3), leading to 4% of enantiopure (*S*)-**19b** and 8% of **19c**. This fact was probably due to sterical hindrance. No amino alcohol compounds were detected in any case. It is important to notice that the 4'-nitro derivative was reduced to the anti-Prelog alcohol (*R*)-**17b**, presenting the same behavior as substrate **9a**.

**Table 4**. Bioreductions of nitroacetophenones catalyzed by *Vigna unguiculata* (t=72 h).[a]




**17**-**19a** (*R*)-**17b** (*S*)-**18**-**19b** **17**-**19c**

**17a** 4'-$NO_2$
**18a** 3'-$NO_2$
**19a** 2'-$NO_2$

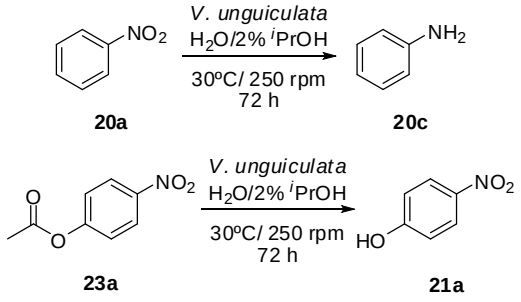
| Entry | Ketone | (%) **17**-**19b**[b] | *ee* (%)[b] | (%) **17**-**19c**[b] |
|---|---|---|---|---|
| 1 | **17a** | 42 | ≥99 (*R*) | 54 |
| 2 | **18a** | 14 | ≥99 (*S*) | 8 |
| 3 | **19a** | 4 | ≥99 (*S*) | 8 |

[a] For reaction conditions, see Experimental Section.

[b] Conversions (*c*) and enantiomeric excesses (*ee*) were calculated by HPLC or GC.

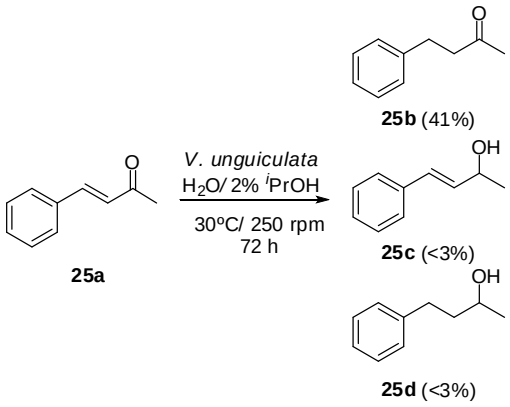
Since we found the unusual nitro reductive activity when reducing nitro ketones **17**-**19a** using whole cells of *V. unguiculata* to obtain the corresponding amines, we

decided to test different aromatic nitro compounds as potential substrates for our biocatalytic system (Scheme 1). When nitrobenzene (**20a**) was treated in the presence of whole cells of *V. unguiculata*, it was possible to obtain 16% of aniline (**20c**) after 72 h. We analyzed 4- (**21a**) and 2-nitrophenol (**22a**) as substrates, but no reaction was observed even after long reaction times, showing that electron-donor substituents disfavored this biotransformation. This fact could explain why amino alcohols were not obtained with nitro ketones **17**-**19a**. 4-Nitrophenyl acetate (**23a**) was also studied, leading after 72 h to the complete formation ($c \geq 99\%$) of the hydrolyzed product 4-nitrophenol (**21a**). When we carried out the blank reaction in the absence of biocatalyst, only 36% of **23a** was hydrolyzed, indicating that *V. unguiculata* was also able to perform the acetate hydrolysis with moderate rate. Benzonitrile (**24a**) was finally employed, but the whole cells were not able to hydrolyze the nitrile moiety.




**Scheme 1**. *V. unguiculata*-catalyzed processes using nitro compounds **20a** and **23a** as substrates in aqueous media containing 2% v $v^{-1}$ $^{i}$PrOH.

Asymmetric alkene reduction is an attractive reaction for organic chemists, as it offers the possibility of creating two adjacent $sp^3$ centers in a single step. Enoate reductases, members of the "*old yellow family*", catalyze this transformation in nature.[15] These enzymes are widely found in microorganisms and plants. By this reason, we have started a preliminary study on this activity employing 4-phenyl-3-buten-2-one **25a** as model substrate in order to determine the ability of *V. unguiculata* to reduce double bonds (Scheme 2). After 72 h, a 41% of 4-phenylbutan-2-one (**25b**) was obtained while only traces (less than 3%) of the other possible reduction products, 4-phenyl-3-buten-2-ol (**25c**) and 4-phenylbutan-2-ol (**25d**), were observed in the reaction media, showing that this activity was present in our preparation.




**Scheme 2**. Bioreduction of 4-phenyl-3-buten-2-one **25a** catalyzed by whole cells of *V. unguiculata* in aqueous media containing 2% v $v^{-1}$ $^{i}$PrOH.

## 3. Conclusions

We have presented *Vigna unguiculata* as an excellent stereo- and chemoselective biocatalyst for the production of interesting organic compounds. Whole cells from this vegetal source have been employed as biocatalytic system applied to different reduction processes. After optimizing the reaction conditions, *i.e.* the amount of cosolvent and the temperature using acetophenone as model substrate, this biocatalyst has shown a remarkable ability to reduce acetophenone derivatives with high to excellent stereoselectivites depending on the substrate structure. In most of the cases, the Prelog reduction products were obtained. Enantiopure (*R*)-β-chloroalcohols, interesting chiral synthons, could be achieved with good conversions. Aliphatic ketones were also reduced with total selectivity, while the bioreduction of β-ketoesters led to the formation of the corresponding enantiopure Prelog β-hydroxyesters with excellent conversions. Furthermore, *Vigna unguiculata* was able to reduce nitro groups to the corresponding amines when, *e.g.* nitrobenzene and nitroacetophenones were employed as substrates, while no reaction was observed in the case of nitrophenols. Whole cells of *V. unguiculata* contained at least one enoate reductase, as this system was able to catalyze preferentially the reduction of the double bond of 4-phenyl-3-buten-2-one with regards to the alcohol formation.

## 4. Experimental

### 4.1. General

Chemical reactions were monitored by analytical TLC, performed on Merck silica gel 60 F254 plates and visualized by UV irradiation. Flash chromatography was carried out with silica gel 60 (230-240 mesh, Merck). IR spectra were recorded on a Perkin-Elmer 1720-X infrared Fourier transform spectrophotometer using KBr pellets. UV spectra were performed on a Perkin Elmer Lambda Bio10

UV/Vis Spectrophotometer. $^{1}$H-NMR, $^{13}$C-NMR and DEPT spectra were recorded with tetramethylsilane (TMS) as the internal standard with a Bruker AC-300 DPX ($^{1}$H: 300.13 MHz; $^{13}$C: 75.5 MHz) spectrometer. $APCI^+$ and $ESI^+$ using a Hewlett Packard 1100 chromatograph mass detector or $EI^+$ with a Hewlett Packard 5973 mass spectrometer were used to record mass spectra (MS). GC analyses were performed on a Hewlett Packard 6890 Series II chromatograph equipped with a Restek RtβDEXse (30 m x 0.25 mm x 0.25 μm, 1 bar $N_2$) or a Varian CP-Chiralsil-DEX CB (25 m x 0.32 mm x 0.25 μm, 1 bar $N_2$) for chiral determinations or a HP-1 (crosslinked methyl siloxane, 30 m x 0.25 mm x 0.25 μm, 1.0 bar $N_2$) from Hewlett-Packard for measuring the conversions values. For all the analyses, the injector temperature is 225ºC and the FID temperature is 250ºC. HPLC analyses were developed with a Hewlett Packard 1100 LC liquid chromatograph equipped with Chiralcel OB-H (0.46 cm x 25 cm), a Chiralpak IA (0.46 cm x 25 cm) or a Chiralpak AS (0.46 cm x 25 cm) chiral columns purchased from Daicel.

Ketones **1**-**4a**, **6a**, **8**-**9a**, **13**-**18a**, **25a**-**b**, alcohol **25d**, nitro compounds **20**-**23a**, aromatic amines **20**-**22c**, and benzonitrile **24a** were products from Sigma-Aldrich, while compounds **5a**, **7a**, **10**-**12a** and **19a** were purchased from Alfa Aesar. All other reagents and solvents were of the highest quality from Sigma-Aldrich-Fluka. All the racemic alcohols (±)-**1**-**19b** and **25d** were prepared by chemical reduction of the corresponding ketones employing $NaBH_4$ in dry methanol at room temperature. In all cases, high yields were achieved (higher than 80%). When necessary, in order to measure the enantiomeric excesses, alcohols were acetylated with acetic anhydride and $Et_3N$ in $CH_2Cl_2$ to obtain the corresponding racemic acetates, being isolated in quantitative yields in all cases. 4-Phenyl-3-buten-2-ol **25c** was prepared following the procedure described in the literature, treating ketone **25a** with $NaBH_4$ in a mixture MeOH-THF 1:10.[16] Aminoketones **17**-**19c** were synthesized by reducing the corresponding nitroketones **17**-**19a** with Sn in water containing concentrated HCl and subsequent treatment with an aqueous solution of NaOH (yields from 60 to 74%).[17]

Absolute configurations of alcohols (*S*)-**1**-**8b**, (*S*)-**13**-**15b**, (*S*)-**18**-**19b**, (*R*)-**9**-**12b** and (*R*)-**16**-**17b** obtained from the *V. unguiculata*-catalyzed reductions were established by comparison of the retention times on GC with previously published data.[11a,11c,18]

### 4.2. Plant material

*V. unguiculata* beans were collected at the planting in rural upstate (Miraíma, CE, Brazil). Bean seeds were rinsed with 5% aqueous solution of sodium hypochlorite and distilled water and dried completely at room temperature. After that, beans were grinded in order to obtain a fine powder that was used as biocatalyst.

### 4.3. General procedure for the *V. unguiculata* biocatalyzed reductions of ketones 1-19a and 25a, nitro derivatives 20-23a and benzonitrile 24a.

In a typical experiment, the substrate **1**-**25a** (5.0 mg) was added to a suspension of *V. unguiculata* powder (1.0 g) in 3.0 mL of distilled water containing 2% v $v^{-1}$ $^{i}$PrOH. Reactions were shaken at 250 r.p.m. at the selected temperatures for the corresponding time. Once finished, the crude reactions were extracted with EtOAc (3 x 5 mL), the organic layers were combined, dried onto $Na_2SO_4$ and the solvent was removed under reduced pressure. Samples obtained were analyzed by GC and/or HPLC in order to determine the conversions and the enantiomeric excesses of the final products.

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