

Biocatalytic Activity of Fresh *Passiflora* Spp. Leaves in Enantioselective Oxido-Reduction

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Abstract

The biocatalytic activity of five species of *Passiflora* leaves (i.e. *P. amethyst*, *P. incarnata*, *P. quadrangularis*, *P. edulis*, *P. cerulea*) was tested in the reduction of the ketone “cocktail” (i.e. 5-hexen-2-one **1**, acetophenone **2**, *cis*-bicyclo[3.2.0]hept-2-en-6-one **3** and 2-methylcyclohexanone **4**) and in the oxidation of the corresponding alcohols “cocktail” (i.e. 5-hexen-2-ol **5**, 1-phenylethanol **6**, *endo*-bicyclo[3.2.0]hept-2-en-6-ol **7**, *exo*-bicyclo[3.2.0]hept-2-en-6-ol **8**, *trans*-2-methylcyclohexanol **9** and *cis*-2-methylcyclohexanol **10**). *P. amethyst* and *P. incarnata* show the best activity in the reduction, while *P. quadrangularis* affords low yield in reduction but gives appreciable results in oxidation towards a cocktail of model substrates. This simple screening permits to test the potential of parts of fresh plants that can be used as biocatalysts in more ecologically and economically promising transformations.

Keywords: *Passiflora amethyst*; *Passiflora incarnate*; *Passiflora quadrangularis*; *Passiflora edulis*; *Passiflora cerulea*; Enantioselective oxido-reduction; Biocatalytic activity

Introduction

Asymmetric synthesis remains a challenge for synthetic chemists as the demand for enantiomerically pure compounds continues to increase. Several of these compounds are potential chiral building blocks of pharmaceutically important molecules, agrochemicals, flavours and asymmetric chiral ligands [1]. Moreover the enantiomers of a number of chiral drugs may exhibit great differences with regard to pharmacodynamics, pharmacokinetics and toxicological properties [2].

In this field the biotransformations, defined as chemical reactions catalysed by isolated enzymes, microorganisms or cell cultures [3], are a “green opportunity” to obtain enantiomerically pure building blocks. The use of purified enzymes [4], whole microorganisms [5] or plant cell cultures [6], mainly at industrial scale, is limited by cost and by the difficulty to handle them, due to their great sensitivity to changes in pH or temperature, requirements of additional cofactors, long and tedious procedures [7]. In order to overcome these limitations, the possible use of vegetable whole organs, as leaves, without any preliminary preparation or transformation, has been considered [8].

Since the biochemical potential of plant cell cultures to produce specific secondary metabolites such as drugs, flavours, pigments and agrochemicals is of considerable interest in connection with their biotechnological utilisation [9], plants have the potential to integrate the greening of organic chemistry with the use of cheap and effective reagents.

Moreover, this methodology offers numerous advantages both in terms of time-saving, 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 [10], and in terms of environment-saving, since the reactions are performed in aqueous media, at room temperature, and generate only biodegradable waste [11].

In a green context, several reports have so far described the possibility of using parts of fresh plants as biocatalysts, i.e. the reduction of aromatic aldehydes using of fresh leaves of banana and maize plants [12], the stereoselective reduction of prochiral ketones by wild tissues of South American endemic plants [13] and the bioreduction of acenaphthenequinones by using peach and carrot [14].

Passiflora commonly known as passion fruit plant belongs to the family Passifloraceae, which comprises nearly 500 species. This genus,

native to Tropical America and Brazil, and widely distributed all over the world [15], is principally popular for their fruits, but, traditionally, in American and European countries leaves are largely used for tea infusion [16]. Machado and co-workers have reported the use of fruit peel of *Passiflora edulis* for the reduction of aromatic aldehydes and ketones, obtaining very good yields and moderate enantioselectivity [17].

In this work, we have screened the biocatalytic activity of various *Passiflora* spp. leaves (i.e., *P. amethyst*, *P. incarnata*, *P. quadrangularis*, *P. edulis*, and *P. cerulea*) using a “cocktail” of ketones (i.e. 5-hexen-2-one **1**, acetophenone **2**, *cis*-bicyclo[3.2.0]hept-2-en-6-one **3**, and 2-methylcyclohexanone **4**) to check the reduction activity, and a “cocktail” of the corresponding racemic alcohols (i.e., 5-hexen-2-ol **5**, 1-phenylethanol **6**, *endo*-bicycloheptenol **7**, *exo*-bicycloheptenol **8**, *trans*-2-methylcyclohexanol **9** and *cis*-2-methylcyclohexanol **10**) to check the oxidation activity (Scheme 1). The use of substrate cocktail has been reported in the activity fingerprint of various hydrolytic enzymes [18] and also the biological activity of thousands natural products and natural product-like molecules have been screened in silico [19-22].

The final research objective was to find new biocatalysts readily accessible from both wild and commercially available natural resources, as a sustainable alternative to traditional chemical methods.

Materials and Methods

General

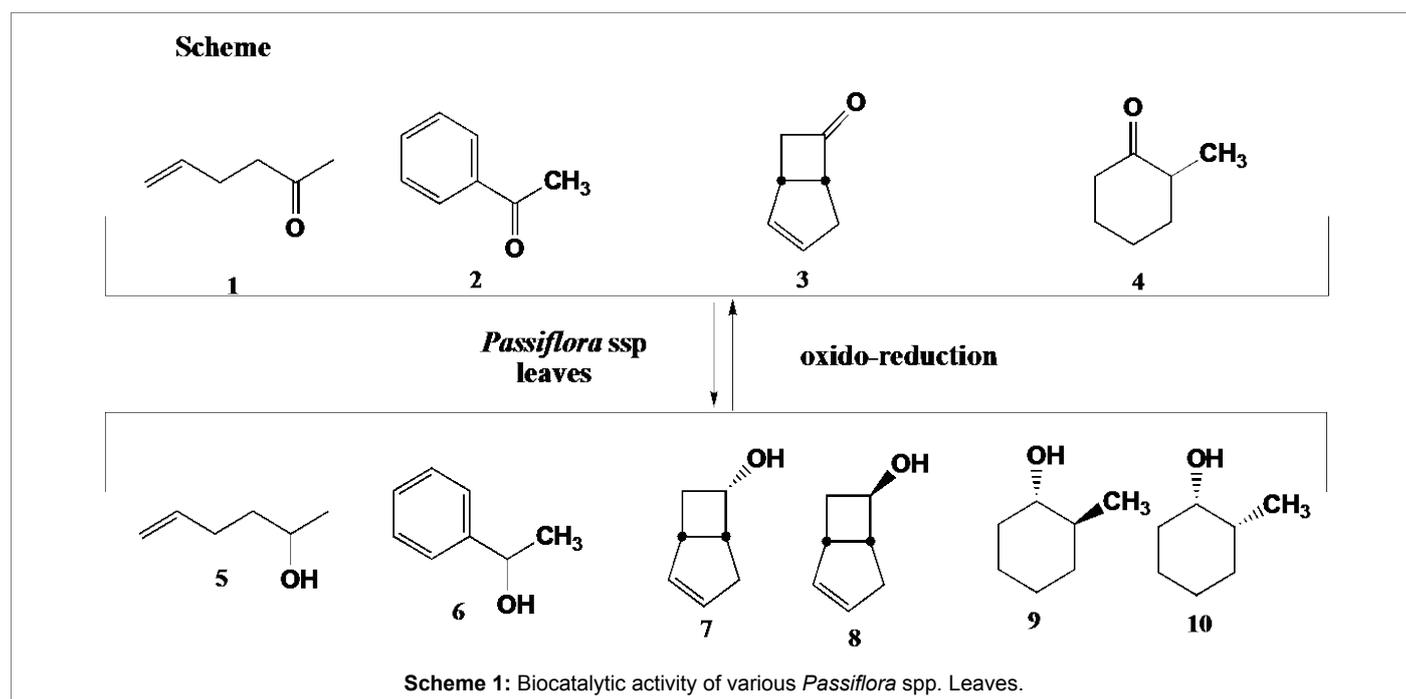
5-Hexen-2-one **1**, acetophenone **2**, *cis*-bicyclo[3.2.0]hept-2-en-6-one **3**, 2-methyl-cyclohexanone **4**, 5-hexen-2-ol **5**, 1-phenylethanol **6**, *trans*-2-methylcyclohexanol **9**, and *cis*-2-methylcyclohexanol **10** are commercially available (Sigma Aldrich[®] Germany). *endo*-Bicyclo[3.2.0]

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hept-2-en-6-ol **7** and *exo*-bicyclo[3.2.0]hept-2-en-6-ol **8** are obtained by reduction of the corresponding ketone **3** with NaBH₄.

Gas chromatographic analyses were performed on a Carlo Erba GC 6000 Vega series 2 with dual Flame-Ionization Detector (FID) (Shimadzu, Japan). The analysis of the reaction mixture was achieved on Megadex DETTBS β column (25 m \times 0.25 mm) containing diethyl-*tert*-butylsilyl β -cyclodextrin in OV 1701; carrier gas: helium 70k Pascal; injector temperature 200°C; detector temperature 250°C; heater temperature 75-80°C (0.5°C/min), 80-100°C (2°C/min), 100-200°C (10°C/min); retention time: **1**, 4.39; (*S*)-**5**, 6.86; (*R*)-**5**, 7.17; (*S*)-**4**, 11.37; (*R*)-**4**, 11.71; (*1R,2R*)-*trans*-**9**, 13.09; (*1S,2S*)-*trans*-**9**, 13.30; (*1S,5R*)-**3**, 13.87; (\pm)-*cis*-**10**, 14.08; (*cis*)-**3**, 16.29; **2**, 18.62; (\pm)-*endo*-**7**, 20.14; (*1S,5R,6R*)-*exo*-**8**, 21.14; (*1R,5S,6S*)-*exo*-**8**, 22.06; (*R*)-**6**, 23.61; (*S*)-**6**, 23.79, biphenyl, 26.7. Biphenyl is used as internal standard (Figure 1).

The ketone cocktail (compounds **1-4**) and the racemic alcohol cocktail (compounds **5-10**) were prepared mixing equal volumes of the solutions (0.1 g in 1 mL of DMSO) of the various compounds.

Biotransformation's with plant leaves general procedure

Five species of fresh *Passiflora* spp. leaves (*P. amethyst*, *P. incarnata*, *P. quadrangularis*, *P. edulis*, and *P. cerulea*) were collected. Samples were rapidly cooled to -80°C to ensure a correct preservation and avoiding foliar tissue degradation, and changes in chemical composition up to be used in the laboratory. Then, leaves were finely cut with a sterilised cutter under a sterile hood, washed with 5% sodium hypochlorite (Sigma Aldrich, Germany) and ethanol, and resuspended in water. To 50 mL of the suspension (1 g or 5 g of leaves), 15 mg of chloramphenicol (Sigma Aldrich, Germany), 1 mL of the appropriate reagent mixtures in DMSO (Merck, Germany) and 0.25 mL of a solution (0.1 g in 1 mL of DMSO) of biphenyl, used as internal standard, were added. The reaction mixtures were stirred on a reciprocator shaker at 150 rpm in the dark at 34°C. An aliquot of each reaction mixture (1 mL) was withdrawn and, after extraction with diethyl ether, analysed by GLC periodically (2, 4, 7, 10, 14, and 21 days). The results of the reduction and of the oxidation are reported in Tables 1 and 2 respectively.

Results and Discussion

The biotransformation procedure was very simple owing the easy availability of plants, the use of water without carbon source and the simple work up, because no emulsion has formed.

Reduction with *Passiflora* spp. leaves

The results of the reduction of the ketone cocktail are reported in Table 1. After 21 days of reduction with all *Passiflora* spp. at 34°C the weight loss was quite high. The percentage of recovered mixture for 5-hexen-2-one **1** was only 30-57%, higher (52-68%) for *cis*-bicyclo[3.2.0]hept-2-en-6-one **3** and 2-methylcyclohexanone **4**, and good (67-95%) for acetophenone **2**.

P. amethyst (1 g) reduced all ketones with good yields (70-94%) but showed poor or no enantioselectivity. Surprisingly the reduction to alcohols decreased (41-71%) with the same leaves (5 g) but the loss of yield is interestingly balanced by the increasing of the ees of the (*R*)-5-hexen-2-ol **5** (ee 27%) and (*S*)-1-phenylethanol **6** (ee 52%). Regarding the reduction of ketones **3** and **4**, only the enantiomeric excesses of the (*1R,5S,6S*)-*exo*-bicycloheptenol **8** and (*1R,2R*)-*trans*-2-methylcyclohexanol **9** were determined because the acetylation of the mixture, that permits the enantiomer separation of compounds **7** [23] and **10** [24], has not been carried out in this preliminary phase.

P. incarnata (1 g) reduced significantly only 5-hexen-2-one **1** and 2-methylcyclohexanone **4** giving the (*S*)-5-hexen-2-ol **5** (52%, ee 46%) and (*1R,2R*)-2-methylcyclohexanol **9** (39%, ee 29%), and *cis*-2-methylcyclohexanol **10** (36%), respectively. On the other hand (*S*)-methylphenyl carbinol **6** (10%, ee 67%), *endo*-bicycloheptenol **7** (15%) and (*1R,5S,6S*)-*exo*-bicycloheptenol **8** (17%, ee 12%) were obtained with low yields. Higher conversion but lower enantioselectivity was obtained with 5 g of the same leaves. Only exception was the reduction of acetophenone **2** that afforded (*S*)-1-phenylethanol **6** (28%, ee 62%).

P. cerulea leaves (1 g) did not reduce 5-hexen-2-one **1** and bicycloheptenone **3** and afforded very low yield of alcohols **6** and **9** (6% and 9%, respectively), while the biotransformation of the ketone

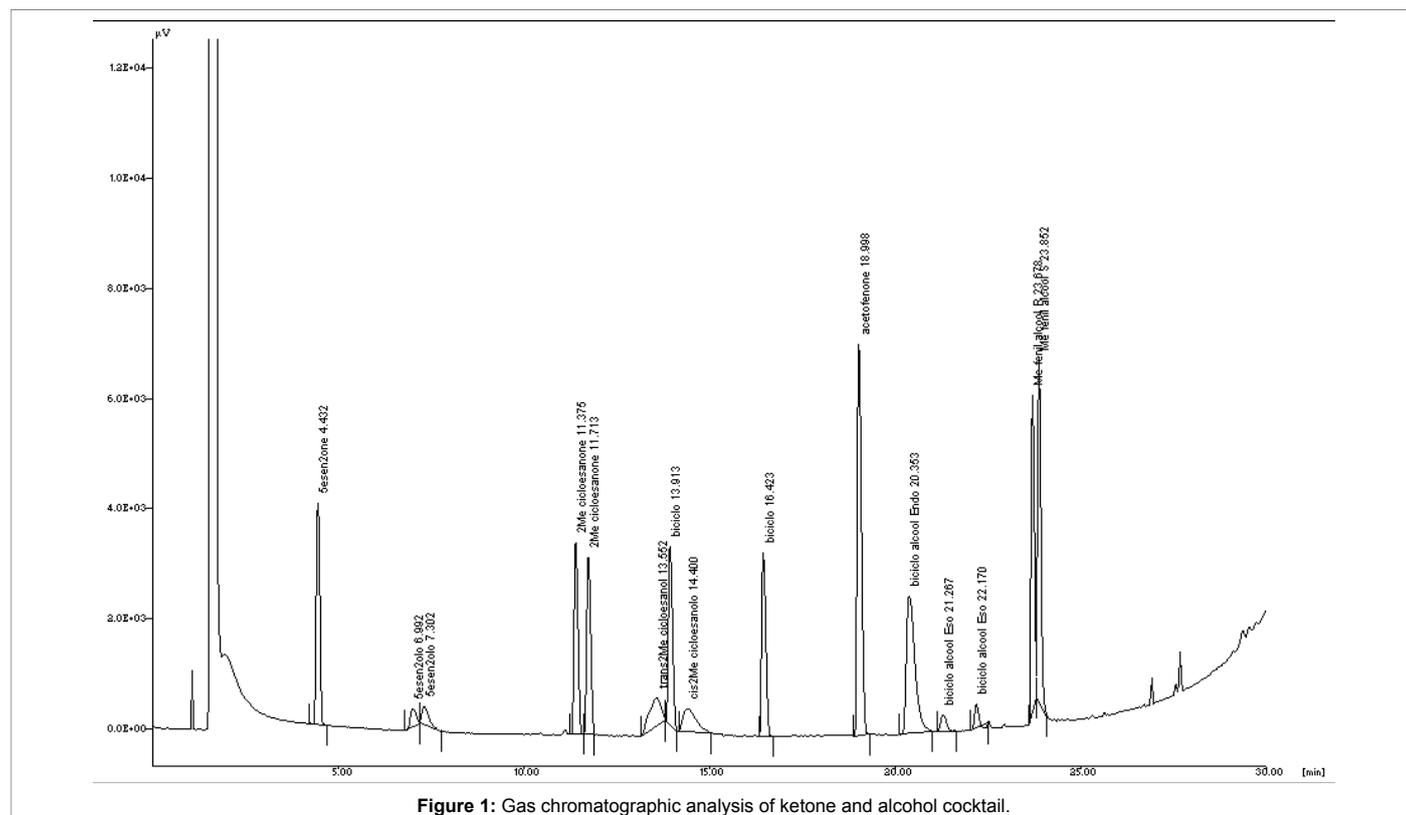


Figure 1: Gas chromatographic analysis of ketone and alcohol cocktail.

<i>Passiflora</i> ssp.	g/L	days	ketone cocktail	recovered mixture ^a	5-hexen-2-ol 5 ^b	1-phenyl-ethanol 6 ^b	endo-bicyclo-heptenol 7 ^c	exo-bicyclo-heptenol (1R,5S,6S)-8 ^b	trans-2-methyl-ciclohexanol (1R,2R)-9 ^b	cis-2-methyl-ciclohexanol 10 ^c
<i>P. amethyst</i>	1	21	1	30	92 (0)	94 (0)	57	26 (21)	45 (5)	25
			2	95						
			3	52						
			4	57						
	5	21	1	56	53 (27-R)	41 (52-S)	10	47 (32)	58 (10)	13
			2	72						
			3	68						
			4	53						
<i>P. incarnata</i>	1	21	1	53	52 (46-S)	10 (67-S)	15	17 (12)	39 (29)	36
			2	68						
			3	55						
			4	60						
	5	21	1	57	86 (9-S)	28 (62-S)	22	42 (0)	46 (9)	38
			2	75						
			3	61						
			4	61						
<i>P. cerulea</i>	1	21	1	30	--	6 (100-S)	--	--	9 (48)	--
			2	63						
			3	48						
			4	46						
	5	21	1	54	14 (25-R)	45 (88-S)	12	11 (41)	44 (30)	18
			2	78						
			3	65						
			4	64						
<i>P. edulis</i>	1	21	1	43	13 (4-S)	3 (60-S)	4	1 (0)	16 (40)	12
			2	74						
			3	62						
			4	60						
<i>P. quadrangularis</i>	1	21	1	49	11 (48-S)	23 (90-S)	8	19 (67)	36 (34)	24
			2	67						
			3	65						
			4	61						

^a Recovered weight percentage of the ketone-alcohol mixture; ^b Yields % and ee% in parenthesis, ^c Yield %

Table 1: Reduction screening of the "cocktail" of the ketones 1-4 with *Passiflora* ssp. Leaves.

<i>Passiflora</i> ssp.	g/L	days	alcohol cocktail	recovered mixture ^a	ketone ^b	5-hexen-2-ol 5 ^c	1-phenylethanol 6 ^c	endo-bicycloheptenol 7 ^d	exo-bicycloheptenol (1 <i>R</i> ,5 <i>S</i> ,6 <i>S</i>)-8 ^c	trans-2-methylcyclohexanol (1 <i>R</i> ,2 <i>R</i>)-9 ^c	cis-2-methylcyclohexanol 10 ^d
<i>P. amethyst</i>	1	14	5 6 7/8 9/10	59 82 56 52	1 (–) 2 (8) 3 (25) 4 (13)	100 (0)	92 (6- <i>S</i>)	52	23(23)	59 (5)	28
	5	14	5 6 7/8 9/10	84 73 49 56	1 (38) 2 (23) 3 (46) 4 (14)	62 (16- <i>S</i>)	77 (29- <i>R</i>)	17	37(25)	60 (49)	26
<i>P. incarnata</i>	1	10	5 6 7/8 9/10	80 76 60 67	1 (50) 2 (7) 3 (41) 4 (11)	50 (47- <i>R</i>)	93 (8- <i>R</i>)	29	30 (7)	49 (39)	40
	5	10	5 6 7/8 9/10	71 64 49 64	1 (35) 2 (24) 3 (52) 4 (17)	65 (28- <i>S</i>)	76 (20- <i>R</i>)	13	35(4)	51 (40)	32
<i>P. cerulea</i>	1	10	5 6 7/8 9/10	53 50 43 44	1 (–) 2 (10) 3 (56) 4 (27)	100 (0)	90 (0)	24	20 (9)	55 (40)	18
	5	10	5 6 7/8 9/10	50 46 42 39	1 (9) 2 (10) 3 (21) 4 (8)	91 (4- <i>R</i>)	90 (0)	62	17 (25)	62 (38)	29
<i>P. edulis</i>	1	10	5 6 7/8 9/10	63 80 68 53	1 (15) 2 (4) 3 (24) 4 (8)	85 (15- <i>R</i>)	96 (3- <i>R</i>)	59	17 (11)	56 (43)	36
<i>P. quadrangularis</i>	1	10	5 6 7/8 9/10	58 73 54 58	1 (26) 2 (40) 3 (49) 4 (31)	74 (8- <i>S</i>)	60 (55- <i>R</i>)	21	30 (49)	43 (48)	26

^aRecovered weight percentage of the ketone-alcohol mixture; ^bYields in parenthesis; ^cYields % and ee% in parenthesis, ^dYield %

Table 2: Oxidation screening of the “cocktail” of the alcohols 5-10 with *Passiflora* ssp. Leaves.

cocktail with 5 g of the same leaves produced (*S*)-1-phenylethanol **6** (45%, ee 88%), (1*R*,2*R*)-2-methylcyclohexanol **9** (44%, ee 30%) and *cis*-methylcyclohexanol **10** (18%) together with low yield of (*R*)-5-hexen-2-ol **5** (14%, ee 25%), *endo*-bicycloheptenol **7** (12%), and (1*R*,2*R*)-2-methylcyclohexanol **9** (11%, ee 41%).

Practically no reduction products were obtained with *P. edulis* leaves (1 g). The only appreciable result was the reduction of 2-methylcyclohexanone **4** to (1*R*,2*R*)-alcohol **9** (16%, ee 40%) and *cis*-alcohol **10** (12%).

Finally, *P. quadrangularis* (1g) gave low yields but good enantiomeric excesses of (*S*)-1-phenylethanol **6** (23%, ee 90%), (1*R*,5*S*,6*S*)-*exo*-bicycloheptenol **8** (19%, ee 67%) and (*S*)-5-hexen-2-ol **5** (11%, ee 48%), together with *endo*-bicycloheptenol **7** (8%). The more interesting result was obtained with 2-methylcyclohexanone **4** that afforded (1*R*,2*R*)-*trans*-alcohol **9** (36%, ee 34%) and *cis*-alcohol **10** (24%).

Oxidation with *Passiflora* spp. leaves

The results of the oxidation of the alcohol cocktail are reported in Table 2. In the case of the oxidation, the weight loss took place, as well. After 10-14 days, the weight of recovered mixture was 50-84% for the alcohols **5** and **6**, and lower (39-67%) for the alcohols **7**, **8**, **9** and **10**.

P. amethyst (5 g) oxidized, after 14 days incubation, the racemic alcohols **5**, **7** and **8** to the corresponding ketones **1** (38%) and **3** (46%), respectively, leaving the (*S*)-5-hexen-2-ol **5** (62%, ee 16%), bicycloheptenols **7** (17%) and (1*R*,5*S*,6*S*)-*exo*-bicycloheptenol **8** (37%,

ee 25%). Low yields (< 25%) were obtained with the other alcohols (**6**, **9** and **10**) and in all cases using 1g of the same leaves.

Also *P. incarnata* (1 g) oxidized with good yields 5-hexen-2-ol **5** and the mixture of bicycloheptenols **7** and **8** to give the corresponding ketones **1** (50%) and **3** (41%) with only a good kinetic resolution of the *R*-alcohol **5** (50%, ee 47%). Similar or worst results were obtained with 5 g of the same leaves.

P. quadrangularis oxidized all the alcohols to the corresponding ketones (26-49% yields) leaving the unreacted (*R*)-1-phenylethanol **6** (60%, ee 55%), (1*R*,5*S*,6*S*)-*exo*-bicycloheptenol **8** (30%, ee 49%) and (1*R*,2*R*)-*trans*-2-methylcyclohexanol **9** (43%, ee 48%). No enantioselectivity was obtained in the oxidation of the alcohol **5**. Finally, the oxidations carried out with *P. cerulea* (1 g) and (5 g), and *P. edulis* (1 g) gave low yields of ketones **1-4** (0-27%) and as much low enantiomeric excesses.

Conclusions

The use of a cocktail of ketones and alcohols allows preliminary information of oxido-reduction potential of *Passiflora* leaves using a simple and fast methodology that permits to test the potential of parts of fresh plants that can be used as biocatalysts in more ecologically and economically promising transformations. *P. amethyst* and *P. incarnata* appear to have a higher inclination towards the reduction with respect to other species, while towards the oxidation there is a greater regularity of behaviour (low yields and enantiomeric excesses) even if *P. amethyst*, *P. incarnata* and *P. quadrangularis* show a higher activity towards the substrate cocktail.

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