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A Comparative Study of the Effect of Various Kinetic Parameters on Asymmetric Bioreduction of Prochiral Ketones Catalyzed by *Phoenix dactylifera* L.

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ABSTRACT

The present work is aimed mainly to investigate and find optimal conditions by changing various kinetic parameters on reaction conversion such as duration of reaction, effect of buffer pH, effect of co-substrate and its concentration, effect of speed of agitation, effect of temperature, effect of substrate concentration, and substrate profile. The enantioselectivity was analyzed systematically over wide range. Among various co-substrates, glucose found to be the best for regeneration of co-factors. These optimal conditions were applied on fundamental and practical asymmetric reduction reaction of prochiral ketones in order to produce non-racemic chiral alcohols, from which many industrially important chemicals such as pharmaceuticals, agrochemicals, and natural products. This study underscored the bioreduction of various acetophenone derivatives. *Phoenix dactylifera L.* was chosen as the biocatalysts and the acetophenone derivatives could be reduced by this plant tissue with high enantioselectivity and only *R*-form configuration chiral alcohols could be obtained. The ee and chemical yield could reach about 89.0 and 68.6% respectively for acetophenone, 79.3 and 34.4% respectively for 4'-nitroacetophenone and (64.0-77.0)% and 44.2-65.5)% respectively for 4'-haloacetophenones (X=F, Cl, and Br). The results obtained in the present study suggest that the Phoenix dactylifera L can be used as biocatalysts in synthesis many enantiomerically pure pharmaceuticals.

Keywords: Biocatalysts, chiral alcohols, acetophenones, Phoenix dactylifera L ,

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INTRODUCTION

The asymmetric reduction of Prochiral ketones is one of the most important, fundamental and practical reaction for producing non-racemic chiral alcohols, from which many industrially important chemicals such as pharmaceuticals, agrochemicals, and natural products. The catalysts for the asymmetric reduction of prochiral ketones can be classified into two categories: chemical and biological methodologies. Presently, there are five chemical reagents which are extensively used in asymmetric reduction: Brown's DIP chloride [1,2], Midlands Alpin-Boranes [3], Corey's oxazaborolidines [4,5], Nyori's BINAL-H and BINAP-Ru complexes [6]. In 2000, L. Sekhri and N. J. Lawrence utilized Corey's oxazaborolidine to obtain excellent yields and enantioselectivities for a variety of phosphynoyl alcohols [7].

In recent years, chemicals reactions using plant cell cultures and part of plants as biocatalysts have received great attention due to the wide biotechnological potential of the enzymatic reactions [8-10]. Some important characteristics of these biocatalysts are their low cost, high versatility and efficiency, in addition to highly desirable chemical aspects such as chemoselectivity, regioselectivity, and enantioselectivity, with the combination of these factors having made biocatalitic reactions very attractive to the industrial sector [11].

The biocatalytic transformations using plants can be applied in bioreduction of ketones [12, 13], enzymatic lactonization [2], hydrolysis of esters [14], addition of hydrogen cyanide [15], and hydroxylation and oxidation reaction [16]. The biocatalysts used for the asymmetric reductions, baker's yeast [17-20], and vegetables [21], germinated plant [22] has been applied to organic synthesis because these biocatalysts are easily obtainable from markets and easily manipulated. An increasing number of reports dealing with the assessment of bioreduction of prochiral ketones using plants are frequently available [23-28].

Following with our studies in asymmetric reduction of prochiral ketones, either using chemical [16] or biological [25] methodologies, we wish to report the study of the effect of various kinetic parameters on reaction conversion in order to obtain the optimal conditions and explore the asymmetric reduction of different kinds of prochiral ketones catalyzed by various plants tissue. Acetophenone **1a** was chosen as a preferred model substrate of simple ketones; 4-chloroacetophenone **2a** was chosen as the model substrate of halogen-containing aromatic ketones such as 4'-bromoacetophenone **3a** and 4'-fluoroacetophenone **4a**, since the single enantiomer of halogen-containing aromatic alcohols is one of the most important kinds of kiral building blocks for many enantiomerically pure pharmaceuticals, such as L-chlorprenaline, R-tomoxetine, S-fluoxetine, R-salbutamol, and R-denopamine [29]. *Phoenix dactylifera L* was chosen as the biocatalyst, since the literature concerning these plants contains little or no information on its uses as biocatalysts.
Phoenix dactylifer L. is known locally as "Djmar".

EXPERIMENTAL

General methods

Acetophenone **1a**, 4'-chloroacetophenone **2a**, 4'-bromooroacetophenone **3a**, 4'-fluoroacetophenone **4a** and 4'-nitroacetophenone **5a**, were purchased from Aldrich. These chemicals were used without further purification. Thin-chromatography (TLC) was performed using precoated plates (Aluminium foil, silica gel 60 F_{254} Merck, 0.25mm). Merck 60 silica gel (230-400 mesh) was used for flash chromatography. Optical rotations were determined on Euromex Polarimeter PM. 5400 (Mitscherlich type polarimeter).

All 300 MHz and 75 MHz ^{13}C NMR spectra were run on a Bruker AC 300 NMR spectrometer. Both ^{1}H NMR and ^{1}C NMR spectra were recorded using $CHCl_3$ as internal standard; Infrared spectra were recorded using a Perkin-Elmer 783 spectrometer equipped with a PE 600 data station.

Biocatalysts

Fresh *Phoenix dactylifer L.* was obtained from a local market. *Phoenix dactylifer L.* was taken from the palm pulp stalks. To increase the contact of the substrate with the biocatalyst, the external pulp of the plants was removed and the rest was carefully cut into small thin pieces (approximately 1 cm long slice).

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Synthesis of the chiral alcohols 1b-5b

The chiral alcohols *1b-5b* were synthesized by reduction of the corresponding acetophenones **1a-5a** using *Phoenix dactylifera L* as biocatalysts.

General procedures for biotransformations:

Typical reaction mixture of (0.02 mol) appropriate ketones **1a-5a (Scheme-.1)**, 3% (W/V) of glucose or i-PrOH (in the case of solid ketones) as a source of hydrogen was added to 25g of *Phoenix dactylifer L*. in 80 mL deionized water. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at 30°C. The progress of the reaction was monitored by TLC. The plants pieces were then removed by filtration, washed with deionized water and the filtrate was extracted with petroleum ether (3x100ml). The petroleum ether fraction was dried over anhydrous ($MgSO_4$) and the solvent was evaporated to get the final product and then chemical yield and enantioselectivity were determined. Each experiment was parallelly repeated at least three times and then the average value and standard deviations were given. The conversion obtained after 24 to 48 hours.The products were identified by comparing their data with those of authentic samples on TLC, by IR, and ^{1}HNMR spectra [30,31]. The presence of alcoholic group in the final product was chemically confirmed by acetyl chloride test.

Determination of optical activity of chiral products:

Optical properties of the products obtained from the prochiral were studied with the help of polarimeter using the method described in our paper reported recently [32].

Identification of chiral alcohols 1b-5b by* optical properties and *spectroscopic data:

Phenylethanol (1b):

(R)-**(1b)** was obtained in 68.6% yield, $[\alpha]_D^t$ +40 (*c* 5, MeOH). The absolute configuration was estimated by analogy with {Lit., [33] $[\alpha]_D^t$ +45 (*c* 5, MeOH) for *R*-isomer}. The IR and ^{1}H and ^{13}C NMR spectra of **(1b)** were identical to those of authentic samples [30,31]. ^{1}H ($CDCl_3$, 300 MHz): δ (ppm): 1.5 (3H, d, $\underline{CH_3}$CHOH-), 4.7 (1H, br.s, $\underline{OH}$), 5.2 (1H, q, -$\underline{CH}$OH), 7.3-7.4 (4H, m, Ar-H); ^{13}C ($CDCl_3$, 75 MHz): δ (ppm): 22.8 ($\underline{CH_3}$CHOH), 69.9 (-$\underline{CH}$OH),), 127.1 (-$\underline{CH, Ar}$), 127.6 (-$\underline{CH, Ar}$), 128.9 (-$\underline{CH}$, Ar), 146.1 (C, Ar); ν_{max} (KBr Disk, Cm^{-1}): 3340-3060 (OH).

4'-Chlorophenylethanol (2b):

(R)-**(2b)** was obtained in 51.5% yield, $[\alpha]_D^t$ +28.5 (*c* 5, EtOH). The absolute configuration was estimated by analogy with {Lit., [33] $[\alpha]_D^t$ +37 (*c* 5, EtOH) for *R*-isomer}. The IR and ^{1}H and ^{13}C NMR spectra of **(2b)** were identical to those of authentic samples [30,31]. (^{1}H ($CDCl_3$, 300 MHz): δ (ppm): 1.3 (3H, d, $\underline{CH_3}$CHOH-), 3.5 (1H, br.s, $\underline{OH}$), 4.7 (1H, q, -$\underline{CH}$OH), 7.0-7.3 (4H, m, Ar-H); ^{13}C ($CDCl_3$, 75 MHz): δ (ppm): 28.08 ($\underline{CH_3}$CHOH), 69.54 (-$\underline{CH}$OH),), 126.93 (-$\underline{CH, Ar}$), 128.25 (-$\underline{CH, Ar}$), 132.94 (C, Ar), 144.44 (C, Ar); ν_{max} (KBr Disk, Cm^{-1}): 3340-3060 (OH).

4'-Bromophenylethanol (3b):

(R)-**(3b)** was obtained in 59.0% yield, $[\alpha]_D^t$ +30 (*c* 5, $CHCl_3$). The absolute configuration was estimated by analogy with {Lit., [33] $[\alpha]_D^t$ +39 (*c* 5, $CHCl_3$) for *R*-isomer}. The IR and ^{1}H and ^{13}C NMR spectra of **(2b)** were identical to those of authentic samples [30,31]. ^{1}H ($CDCl_3$, 300 MHz): δ (ppm): 1.5 (3H, d, $\underline{CH_3}$CHOH-), 4.7 (1H, q, -$\underline{CH}$OH), 5.2 (1H, br.s, $\underline{OH}$), 7.3-7.9 (4H, m, Ar-H); ^{13}C ($CDCl_3$, 75 MHz): δ (ppm): 28.03 ($\underline{CH_3}$CHOH), 69.54 (-$\underline{CH}$OH),), 126.93 (-$\underline{CH, Ar}$), 128.25 (-$\underline{CH}$, Ar), 132.94 (C, Ar), 144.44 (C, Ar); ν_{max} (KBr Disk, Cm^{-1}): 3340-3060 (OH).

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4'-Fluorophenylethanol (4b):

(R)-**(4b)** was obtained in 55.5% yield, $[\alpha]_D^t$ +40 (*c* 5, $CHCl_3$). The absolute configuration was estimated by analogy with {Lit., [33] $[\alpha]_D^t$ +50 (*c* 5, $CHCl_3$) for *R*-isomer}. The IR and ^1H and ^{13}C NMR spectra of **(2b)** were identical to those of authentic samples [30,31]. ^1H ($CDCl_3$, 300 MHz): δ (ppm): 1.4 (3H, d, $\underline{CH_3}CHOH$-), 3.2 (1H, br.s, OH), 4.8 (1H, q, -CHOH), 6.8-7.0 (2H, m, Ar-H), 7.1-7.3 (2H, m, Ar-H); ^{13}C ($CDCl_3$, 75 MHz): δ (ppm): 22.8 ($\underline{CH_3}CHOH$), 69.9 (-CHOH),), 115.7 (-CH, Ar), 126.9 (-CH, Ar), 141.7 (C, Ar), 161.8 (C, Ar); ν_{max} (KBr Disk, Cm^{-1}): 3340-3060 (OH).

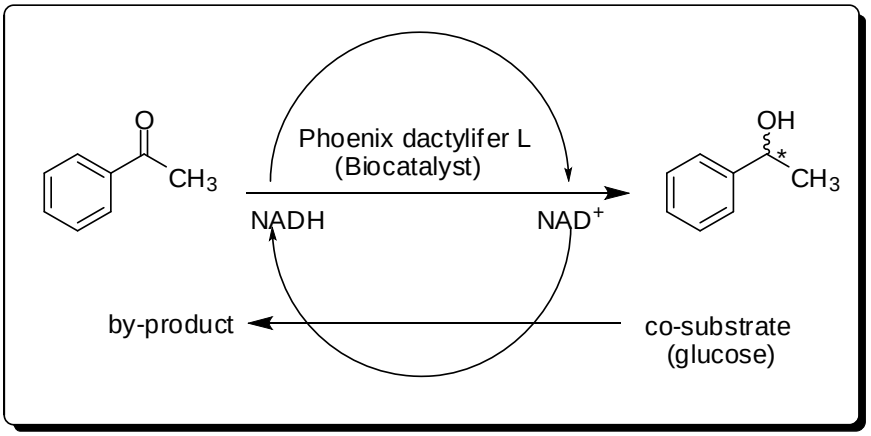
4'-Nitrophenylethanol (5b):

(R)-**(5b)** was obtained in 52% yield, $[\alpha]_D^t$ +30 (*c* 5, MeOH). The absolute configuration was estimated by analogy with {Lit., [33] $[\alpha]_D^t$ +31(*c* 5, MeOH) for *R*-isomer}. The IR and ^1H and ^{13}C NMR spectra of **(5b)** were identical to those of authentic samples [30,31]. ^1H ($CDCl_3$, 300 MHz): δ (ppm): 1.4 (3H, d, $\underline{CH_3}CHOH$-), 2.6 (1H, br.s, OH), 4.9 (1H, q, -CHOH), 7.4 (2H, d, Ar-H), 8.1 (2H, d, Ar-H); ^{13}C ($CDCl_3$, 75 MHz): δ (ppm): 25.27 (CH_3), 69.27 (-CHOH),), 123.56 (-CH, Ar), 126.04 (-CH, Ar), 146.89 (C, Ar), 153.23 (C, Ar); ν_{max} (KBr Disk, Cm^{-1}): 3340-3060 (OH).

RESULTS AND DISCUSSION

The strategy we have adopted for this asymmetric reduction consists of the following steps:

I. It was therefore decided in the first step to test whether the proketones could undrgo the asymmetric reduction by using *Phoenix dactylifer L* as biocatalyst, acetophenone and glucose as a source of hydrogen according to **Scheme-1** in the following reaction:



Scheme .1. Bioreduction of acetophenone catalyzed by Phoenix dactylifera L.

The reaction was carried out according to our procedure rported recently [33]. The reaction mixture of (4 ml) acetophenone, 5% (W/V) of glucose, 20 ml of phosphate buffer (pH = 6.5) was added to 20g of *Phoenix dactylifer L* in 80 mL deionized water. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at 25°C for two days. The yield and enantiomeric excess were 65 and 70% respectively.

II. In the second step, the experiment was repeated with changing various kinetic parameters on reaction conversion such as duration of reaction, effect of buffer pH, effect of co-substrate and its concentration, effect of speed of agitation, effect of temperature, effect of substrate concentration, and substrate profile. The enantioselectivity was analyzed systematically over wide range.

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Effect of reaction duration:

The effect of duration of asymmetric reduction was studied using (4 ml) acetophenone, 5% (W/V) of glucose as a source of hydrogen, 20 ml of phosphate buffer (pH = 6.5), 20g of *Phoenix dactylifer L* and 80 mL deionized water at 25°C **(Fig.1)** according to the procedure described earlier. The results are summarized in **table .1**.

Table .1: The effect of Duration of reaction on biocatalytic activity of *Phoenix dactylifer L*

Phoenix dactylifer L (g)	Acetophenone (ml)	co-substrate (glucose) (ml)	Time (day)	Yield (%)	Enantiomeric excess (ee%)
20	4	5%	1/2(12h)	5	-----
20	4	5%	2	68.6	89
20	4	5%	4	65	81
20	4	5%	6	67	79

The conversion increased gradually with reaction time and attained maximum level at 2 days. There was no significant change in enantiomeric excess observed after certain time. The results can be represented in the **Fig. .1**

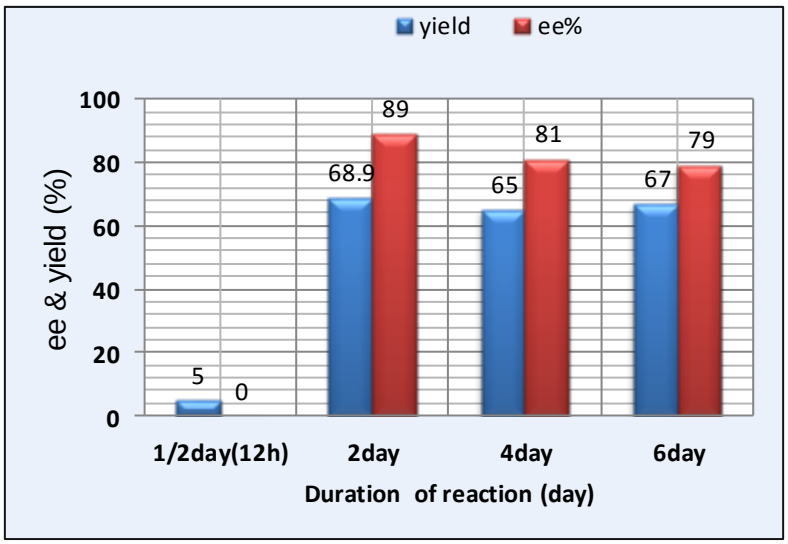

Fig.1: Effect of duration on the yield and ee% for asymmetric reduction of acetophenone

Effect of buffer pH:

The effect of pH on biocatalytic activity of *Phoenix dactylifer L*.was studied by changing pH in range of (5.8 -8.0) and the solutions were prepared as shown in **table.2.** Thus, the reaction mixture of (4 ml) acetophenone, 5% (W/V) of glucose, was added to 20g of *Phoenix dactylifer L* in 80 mL deionized water. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at 25°C for two days.

Table .2.: Solution buffer

pH.25°C	5.8	6.5	7.4	8.0
x.ml 0.2M Na_2HPO_4	4.0	13.25	40.5	47.35
y.ml 0.2M NaH_2PO_4	46	36.75	9.5	2.65

The yield and enantiomeric excess were 60 and 70% respectively. The experiment was repeated with changing by changing pH on reaction conversion. It is well known that buffer pH will change the activity and stability by altering ionic stable of protein molecules that lead to change in 3D conformational structure of

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enzyme. Thus pH could control the conversion, rate and enantiomeric excess to a large extent. The conversion and rate of reaction increased with increase in pH and maximum conversion was reached at pH 6.5 after which the conversion started to decrease with increase in pH. The results are summarized in **table 3.**

Table 3: The effect of pH on biocatalytic activity of *Phoenix dactylifer L*

Phoenix dactylifer L (g)	Acetophenone (ml)	co-substrate (glucose) (ml)	buffer pH	Yield (%)	Enantiomeric excess (ee%)
20	4	5%	5.8	50	30
20	4	5%	6.5	60	70
20	4	5%	7.4	53	59
20	4	5%	8.0	20	45

The conversion increased gradually with reaction time and attained maximum level at pH=6.5. There was no significant change in enantiomeric excess observed after certain pH. The results can be represented in the **Fig. 2.**

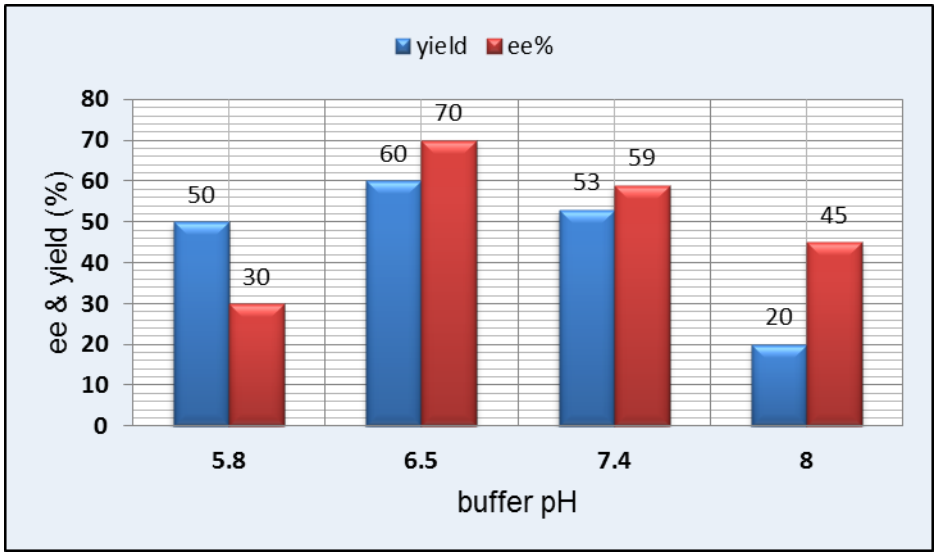

Fig. 2: Effect of buffer pH on the yield and ee% for asymmetric reduction of acetophenone

Effect of co-substrate and its concentration

The effect of co-substrate on biocatalytic activity of *Phoenix dactylifer L.*was studied by changing co-substrate fro i-Propanol, EtOH, Glucose, and sacarose. The reaction mixture of (4 ml) acetophenone, 5% (W/V) of substrate, 20 ml of phosphate buffer (pH = 6.5) was added to 20g of *Phoenix dactylifer L* in 80 mL deionized water. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at 25°C for two days. The yield and enantiomeric excess were 70 and 60% respectively. The results are summarized in **table 4**.

Table 4: The effect of co-substrate on biocatalytic activity of *Phoenix dactylifer L*

Phoenix dactylifer L (g)	Acetophenone (ml)	co-substrate	Yield (%)	Enantiomeric excess (ee%)
20	4	-----	-----	-----
20	4	*i*-Propanol	45	57
20	4	Ethanol	40	45
20	4	Glucose	70	60
20	4	sacarose	50	53

Co-factors like NAD(P)H and FADH play important role in oxidation-reduction reactions catalyzed by reductase/dehydrogenases. Generally small chain alcohols such as ethanol, propanol, isopropanol or glucose have been used**.** The results can be represented in the **Fig. 3.**

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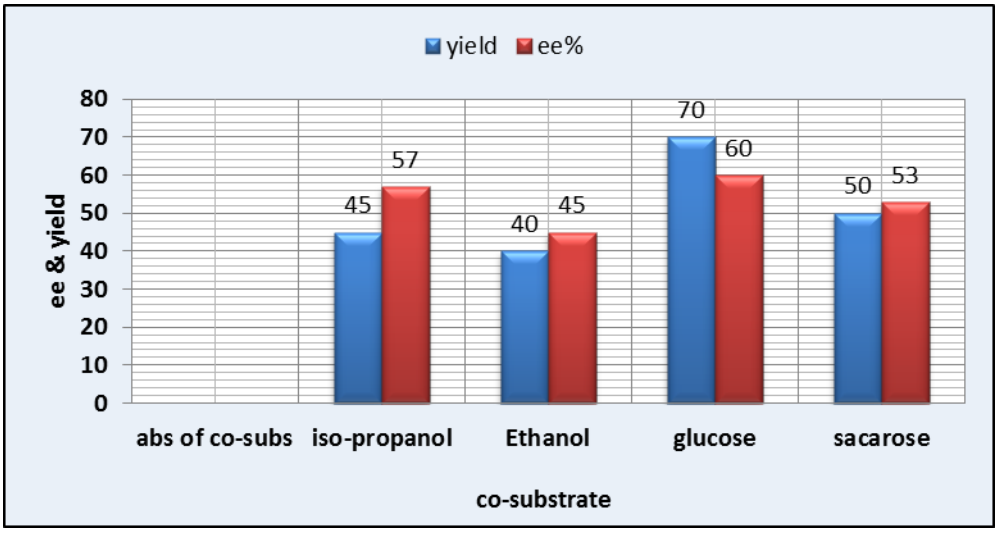

Fig. 3: Effect of co-substrate on the yield and ee% for asymmetric reduction of acetophenone

Effect of glucose concentration

Among various co-substrates, glucose found to be the best for regeneration of co-factors**.** It was therefore decided to study the effect of its concentration on the yield and ee% for asymmetric reduction of acetophenone and the results are shown in **table 4**.

Table.4: The effect of glucose concentration on biocatalytic activity of *Phoenix dactylifer L*

Phoenix dactylifer L (g)	Acetophenone (ml)	co-substrate (glucose) (3%v/w)	Time (day)	Yield (%)	Enantiomeric excess (ee%)
20	**4**	**1%**	**2**	**50**	**71**
20	**4**	**1.5%**	**2**	**52**	**70**
20	**4**	**2%**	**2**	**67**	**75**
20	**4**	**3%**	**2**	**71**	**70**
20	**4**	**5%**	**2**	**67**	**69**

The yield and enantiomeric excess were 71 and 70% respectively and the results can be represented in the **Fig.4.**

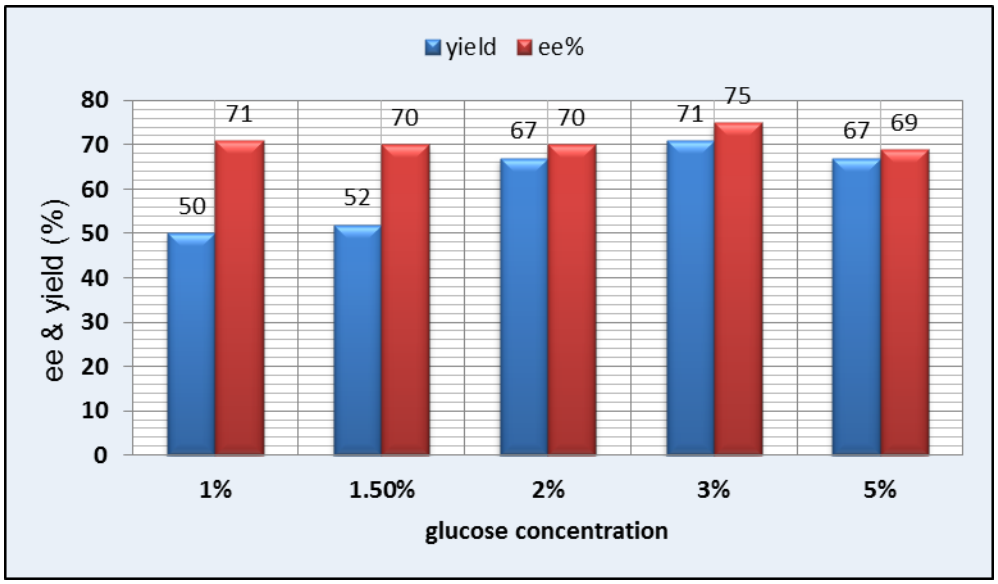

Fig 4: Effect of glucose concentration on the yield and ee% for asymmetric reduction of acetophenone.

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Effect of temperature

Since temperature is one of the parameters with remarkable influence on the conversion and reaction rate of any biocatalytic reactions, The Effect of temperature on asymmetric reduction was studied using (4 ml) acetophenone, 5% (W/V) of glucose as a source of hydrogen, 20g of *Phoenix dactylifer L* and 80 mL deionized water at. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at (25° to 50°C) for two days according to the procedure described earlier. The results are summarized in **table 5**.

Table 5: The effect of temperature on biocatalytic activity of *Phoenix dactylifer L*

Phoenix dactylifer L (g)	Acetophenone (ml)	co-substrate (glucose) (5%v/w)	Temperature (°C)	Yield (%)	Enantiomeric excess (ee%)
20	**4**	**5%**	**25**	**60**	**73**
20	**4**	**5%**	**30**	**71**	**81**
20	**4**	**5%**	**40**	**15**	**--**
20	**4**	**5%**	**50**	**10**	**--**

The yield and enantiomeric excess at 30°C were 71 and 81% respectively and the results can be represented in the **Fig.5.** There was no considerable change in conversion observed above 30 °C. The enantiomeric excess of alcohol was not changed significantly over the temperature range studied here. Most of the reported microbial and plant based whole cells have shown significant loss of activity at temperatures above 35 °C.

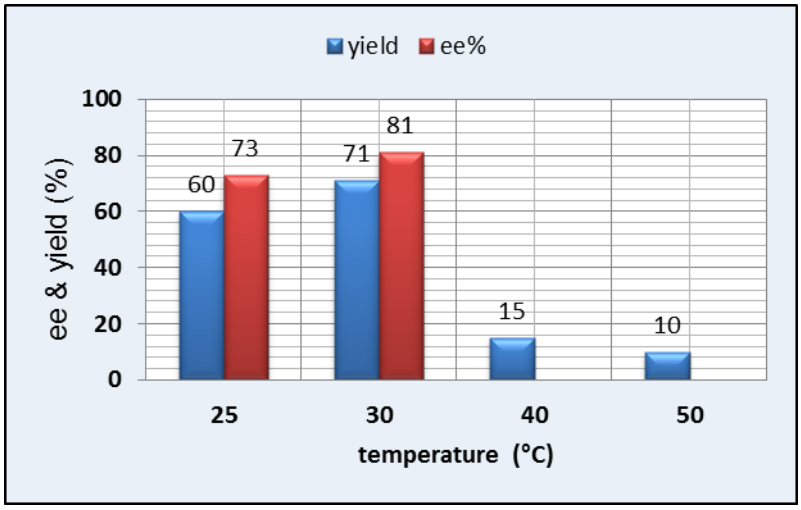

Fig. 5: Effect of temperature on the yield and ee% for asymmetric reduction of acetophenone.

Effect of Biocatalyst (*Phoenix dactylifer L.*) concentration

The reaction was carried out at appropriate amount of biocatalyst (*Phoenix dactylifer L.*) to study the intrinsic kinetics of reaction. Thus, experiment was performed using a mixture of (4 ml) acetophenone, 5% (W/V) of glucose, was added to (15-30g) of *Phoenix dactylifer L* in 80 mL deionized water. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at 30°C for two days as described in the earier procedure, and we obtained the results shown in the **table 6.**

Table 6: The Effect of *Phoenix dactylifer L.* concentration on bioreduction of acetophenone

Phoenix dactylifer L (g)	Acetophenone (ml)	co-substrate (glucose, v/w)	Yield (%)	Enantiomeric excess (ee%)
15	**4**	**5%**	**30**	**70**
20	**4**	**5%**	**66**	**79**
25	**4**	**5%**	**70**	**76**
30	**4**	**5%**	**65**	**73**

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A number of experiments were performed in the range from 15 to 30 g of *Phoenix dactylifer L.* under similar conditions**.** The conversion and rate of reaction increased with increase in biocatalyst up to 20g. Initially, the increase of biocatalyst markedly enhances the reaction rate and hence the conversion. However, at high biocatalyst, number of enzyme molecules available for reaction exceeded the number of substrate molecules and hence there were no significant changes in initial rate and conversion of reaction. Further reaction studies were carried out using 20g. The yield and enantiomeric excess were 66 and 79% respectively and the results can be represented in the **Fig 6.**

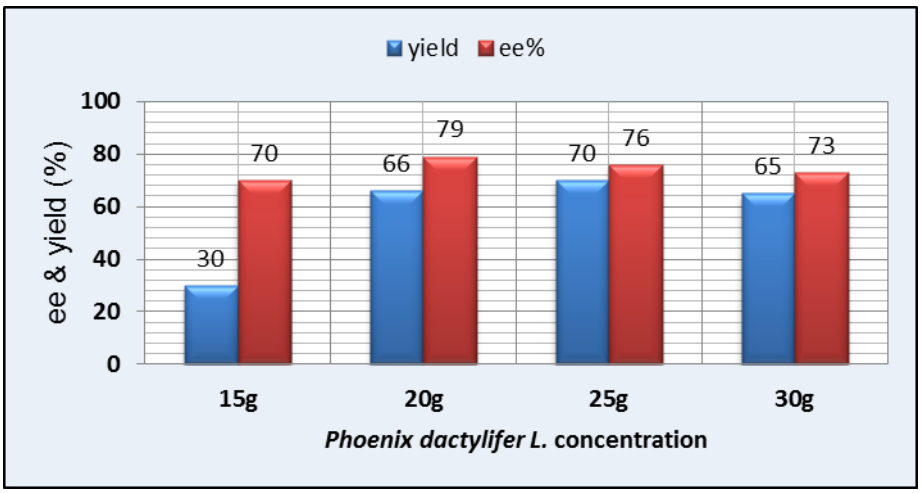

Fig. 6: Effect of *Phoenix dactylifer L.* concentration on the yield and ee% for asymmetric reduction of acetophenone.

Effect of substrate concentration

The reaction was carried out at appropriate amount of substrate (*acetophenone*) to study the intrinsic kinetics of reaction. Thus, experiment was performed using a mixture of (0.02-0.06 mol) acetophenone, 5% (W/V) of glucose as a source of hydrogen, was added to 25g of *Phoenix dactylifer L* in 80 mL deionized water. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at 30°C for two days as described in the earlier procedure, and we obtained the results as shown in the **table 7.**

Table 7: The effect of substrate concentration on biocatalytic activity of *Phoenix dactylifer L*

Phoenix dactylifer L (g)	Acetophenone (mol/ml)	co-substrate (glucose, v/w)	Yield (%)	Enantiomeric excess (ee%)
20	**0.02**	**5%**	**80**	**73**
20	**0.03**	**5%**	**71**	**70**
20	**0.05**	**5%**	**30**	**68**
20	**0.06**	**5%**	**10**	**--**

It has been reported that one of the major limitations of asymmetric reduction of prochiral ketone catalyzed by whole cell biocatalyst was severe toxicity caused by aromatic ketones to microorganisms. Because of this, reduction reactions were generally conducted with low substrate concentration in the range of 0.02-0.06 mol. Further the initial substrate concentration affected the rate and selectivity of reaction because cells contain different kind of reductases which have different affinity towards substrate. The yield and enantiomeric excess were 80 and 73% respectively and the results can be represented in the **Fig. 7.**

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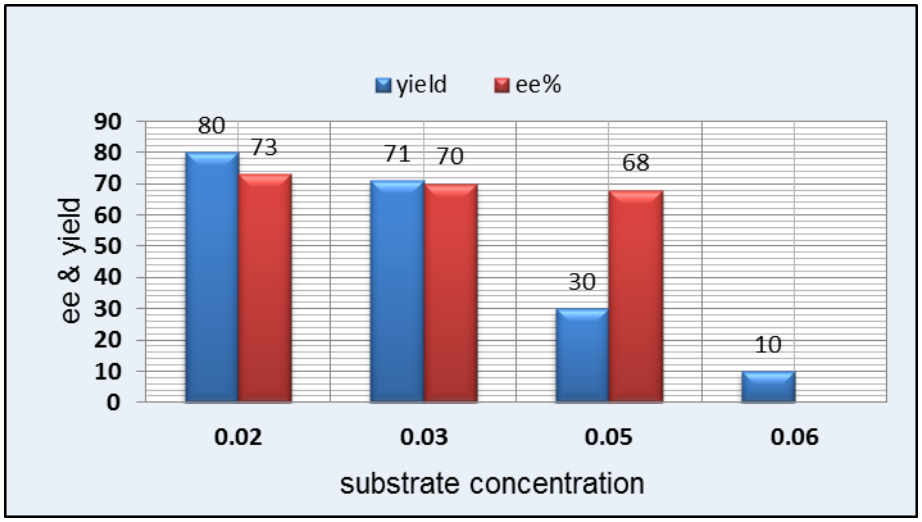
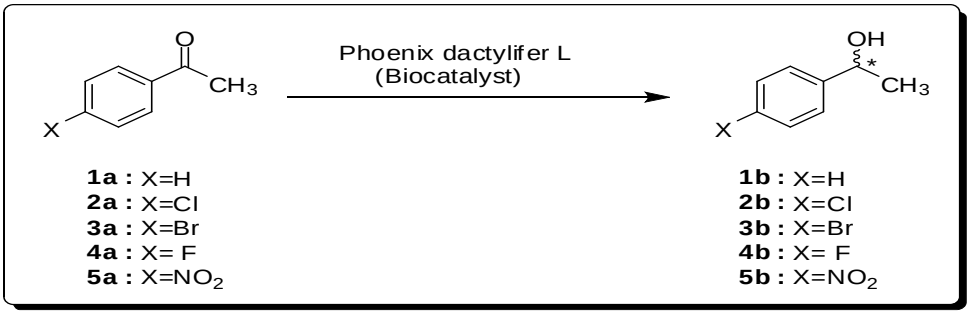


Fig. 7: Effect of substrate concentration on the yield and ee% for asymmetric reduction of acetophenone.

Bioreduction of acetophenone derivatives

Different substituted acetophenones were used as substrate to evaluate the effect of different functional group bearing ketones on asymmetric reduction using *Phoenix dactylifer L*. as biocatalyst under the previous optimized conditions **(Scheme-2).**



Scheme .2. Bioreduction of acetophenone derivatives

For all the cases, *Phoenix dactylifer L* have given good conversion and enantioselectivity (enantiomeric excess was 60 to 89%) and the product alcohol had R-stereospecificity **Table.8).**

Table .8. Bioreduction of acetophenone derivatives by *phoenix dactylifera L*

Resulting alcohols	Time (day)	Yield (%)	ee (%)	$[\alpha]_D^t$ ($dm^{-1}.g^{-1}.ml^{-1}$)	$[\alpha]_D^t$ (pure enantiomer)	Config.
1b	**2**	**68.6**	**89.0**	**+40.0**	**(*R*)+45 (c=5 in MeOH)**	***R***
	4	**77.2**	**75.5**	**+34.0**		
2b	**2**	**44.2**	**62.0**	**+22.8**	**(*R*)+37 (c=0.7 in EtOH)**	***R***
	4	**51.5**	**77.0**	**+28.5**		
3b	**2**	**59.0**	**77.0**	**+30.0**	**(*R*)+39 (c=1 in $CHCl_3$)**	***R***
	4	**65.5**	**64.0**	**+25.0**		
4b	**2**	**55.5**	**80.0**	**+40.0**	**(*R*)+50 (c=1 in $CHCl_3$)**	***R***
	4	**59.4**	**60.0**	**+30.0**		
5b	**2**	**34.4**	**79.3**	**+30.0**	**(*R*)+31 (c=1.2 in MeOH)**	***R***
	4	**52.0**	**82.0**	**+30.0**		

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CONCLUSION

This study underscored the investigation and finding the optimal conditions by changing various kinetic parameters on reaction conversion such as the effect of reaction duration, effect of buffer pH, effect of co-substrate and its concentration, effect of speed of agitation, effect of temperature, effect of substrate concentration, and substrate profile. The enantioselectivity was analyzed systematically over wide range. Among various co-substrates, glucose found to be the best for regeneration of co-factors. These optimal conditions were applied on asymmetric reduction reaction in order to produce non-racemic chiral bioreduction of various acetophenone derivatives with *Phoenix dactylifer L.* Acetophenone, 4'-haloacetophenones (X=F, Cl, and Br) and 4'-ntroacetophenone can be effectively reduced to the corresponding chiral alcohols with the yield and ee are (34.4-77.2) and (60.0-89.0) % respectively and the reaction reached the equilibrium within 2-4 days (48-96 hours). Moreover, only *R*-form configuration chiral alcohols could be obtained through these asymmetric reduction reactions catalysed by *Phoenix dactylifer L* and S- configuration of these alcohols **1b-5b** were confirmed by comparison of their specific rotation with those of described in the literature [34], what is in agreement with Prelog model for bioreduction [35]. This provides a new rout to produce chiral alcohols, as the platform chemicals for enantiomerically pure pharmaceuticals, through asymmetric reduction of the corresponding prochiral ketones.

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REFERENCES

[1] Orden, A. A., Bisogno, F. R., Giordano, O.S., Sanz, M. K., J. Mol. Catal. Enzyme., (2008) 51: 49-55.
[2] Olejniczak, T., Mironowicz, A., Wawwrzenczyk, C., Bioorg., Chem., (2003) 31: 109-116.
[3] Midland, M. M., Chem. Rev., (1989) 89: 1553-1561.
[4] Corey, E. J., Bakshi, R. K., Shibata, S., J. Am. Chem. Soc., (1987) 109: 5553-5561.
[5] Corey, E. J., Bakshi, R. K., Shibata, S., Singh, V. K., J. Am. Chem. Soc., (1987) 109: 7925-7931.
[6] Henry, B. K., Maurice, T., Jean, C. F., Tetrahedron Lett., (1991) 42: 4959-468.
[7] Sekhri, L., Lawrence, N. J., J. Soc. Alger. Chim., (2000) 10: 9-21.
[8] Giri, A., Dhinga, V., Giri, C. C., Singh, A., Ward, O. P., Narsu, M. L., Biotechnol. Adv., (2001) 19: 175-185.
[9] Villa, R., Molinari, F., Levati, M., Aragozzini, F., Biotechnol. Lett., (1998) 20: 1105-1112.
[10] Bruni, R., Fantin, G., Medici, A., Pedrini, P., Sacchetti, G., Tetrahedron Lett., (2002) 43: 3377-3385.
[11] Cordell, G. A., Lemos, T.L.G., Monte, F.G.Q., de Matos, M.C., Nat Prod, J., (2007) 70: 478-492.
[12] Machado, L.L., Souza, J.S.N., Mattos, M.C., Sakata, S.K., Cordell, G.A., Lemos, T.L.G., Phytochemistry, (2006) 67: 1637-1643.
[13] Rodriguez, P., Barton, M., Aldabalde, V., Onetto, S., Panizza, P., Menendez, P., Gonzalez, D., Rodriguez, S., J. Mol. Catal. Enzyme, (2007) 49: 8-11.
[14] Maczka, W. K., Tetrahedron: Asymm., (2002) 13: 2299-2305.
[15] Hamandez, L., Luna, H., Ruiz-Teran, F., Vasquez, A., J. Mol. Catal. B: Enzyme, (2004) 30:105-112.
[16] Sakamaki, H., Itoh, K., Taniai, T., Kitanaka, S., Takgi, Y., Chai, W., Horiuchi, C.A., J. Mol. Catal. B: Enzyme, (2005) 32: 103-110.
[17] Yasohara, Y., Kizaki, N., Hasegawa, J., Takahashi, S., Wada, M., Katoka, M., Shimizu, S., Appl. Microbial. Biotechnol., (1999) 51: 847-845.
[18] Wada, M., Katoka, M., Kawabata, H.,Yasohara, Y., Kizaki, N., Hasegawa, J., Takahashi, S., Shimizu, S., BioSc. Biotech. Biochem., (1999) 62: 280-288.
[19] Ferabochi, P., Grisenti, P., Manzocchi, A., Santaniello, E., J. Chem. Soc., Perkin, (1990) 1: 2469-2480.
[20] Ramaswamy, S., Oehischlager, A. C., Tetrahedron, (1991) 47: 1145-1152.
[21] Utsukihara, T., Watanabe, S., Tomiyama, A., Chai, W., Horiuchi, C. A., J. Mol. Catal. Enzyme, (2006) 41: 103-109.
[22] Matsuo, K., Kawabe, S., Tokuda, Y., Eguchi, T., Yamanaka, R., Nakumera, K., Tetrahedron: Asummetry, (2008) 19: 157-168.
[23] Phukan, K., Devi, N., International Journal of ChemTech Reasearch, (2012) 4(1) : 203-207.
[24] Matsuo, K., Kawabe, S., Tokuda, Y., Eguchi, T., Yamanaka, R., Nakumera, K., Tetrahedron: Asymmetry, (2008) 19: 157-159.
[25] Sekhri, L., Nedjimi, M., Biomedical and Pharmacology Journal, (2009) 2(2): 287-292.

[26] Yang, Z. H. Zeng, R. Yang, G., Wang, Y., Zhen Li, L., Zao-Sheng, Lv. Yao, M., Lai, B., J Ind Microbiol Biotechnol, (2008) 35: 1047-1051.
[27] Andrade, L. H., Utsunomiya, R. S., Omori, A. T., Porto, A. L. M., Comasseto, J.V., J. Journal of molecular Catalysis B: Enzymatic, (2006) 38: 84-90.
[28] Selvi, N. A., Chattopadhyay, S., Tetrahedron, (2001) 57: 2833-2839.
[29] M. Serbnik, P. V. Ramachandran, H. C. Brown, Org Chem., 53(1988) 2916-2920.
[30] Sekhri, L., PhD thesis, Constantine University, Algeria, 1998.
[31] Drew, M. D., Fontaine, D., Lawrence, N.J., Sekhri, L., Synlett., (1997): 989-991.
[32] Nedjimi, M. S., Sekhri, L., Biomedical and Pharmacology Journal, (2016) 9 (1): In press.
[33] Aldrich Catalogue Handbook of fine Chemicals, 1995/1996.
[34] Yadav, J. S. Nanda, S., Reddy, P.T., Rao, A.B., J. Org. Chem., (2002) 67: 3900-3903.
[35] Vries, E.F.J., Brussee, J., Kruse, C.G., Van der Gen, A., Tetrahedron: Asymmetry, (1994) 5 : 377-381.