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Biocatalytic Preparative Methods of Asymmetric Alcohols using Figs (*Ficus carica*)

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ABSTRACT

*A general, efficient and simple methodology for biocatalytic reduction of carbonyl compounds at room temperature using freshly cut ripen fruit of **figs (Ficus carica)** in aqueous medium, has been reported. It was found that the prochiral ketones could be reduced to chial secondary alcohols in a generalized way. The obtained results indicated that **figs (Ficus carica)** fruits can be used as biochemical catalysts to contribute to the preparation of many pharmaceutical compounds. This biochemical catalyst attracted much attention because of the low cost, high efficiency and special selectivity for its environmental friendliness and its contribution to certain recommended green chemistry principles. The aim of this research was to contribute to this area by using biochemical catalysts with plant sources such as **figs (Ficus carica)** fruits by different states (fresh, Juice, dried**). The prochiral ketones: acetophenone was chosen as typical ketone, the yield was (78-84%), and the optical purity was (50-96%). Mild reaction condition, simple operation, and easy availability of **figs (Ficus carica)** fruit revealed this protocol as an attractive and alternative eco-friendly option for general reduction of all types of carbonyl compounds.*

Keywords: *Ficus carica, Figs, biocatalyst, asymmetric reduction, chiral alcohols.*

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1. INTRODUCTION

Many of the plants and vegetables have been used as biochemical catalysts in organic preparation instead of chemicals such as *Cynara scolymus L, Terfezia sp and Phoenix dactylifera L.* fruit *(Mespilus germanica L)* (Nedjimi, Sekhri et al., RJPBCS 2016; Nedjimi, Sekhri et al., Biomedical 2016; Bennamane et al., 2014; Matsuo et al., 2008; Nakumera et al., 2003).

In recent years, the interactions with plant-based enzymes have generated a great deal of interest in their vast biotechnological potential (Bruni et al., 2002; Villa et al., 1998; Utsukihara et al., 2006; Giri et al., 2001), some of the important features of these biomarkers have been their low cost, high versatility and efficiency, as well as highly desirable chemical aspects such as chemical fusion, which made the biochemical reactions very attractive for the industrial sector (Cordell et al., 2007). The biocatalytic transformations using plants can be applied in bioreduction of ketones (Machado et al., 2006; Rodriguez et al., 2007), enzymatic lactonization (Olejniczak et al., 2003), hydrolysis of esters (Maczka et al., 2002), addition of hydrogen cyanide (Hamandez et al., 2004), and hydroxylation and oxidation reaction (Sakamaki et al., 2005). The biocatalysts used for the asymmetric reductions, baker's yeast (Yasohara et al., 1999; Wada et al., 1999; Ferabochi et al., 1990; Ramaswamy et al., 1991), and vegetables (Utsukihara et al., 2006), germinated plant (Matsuo et al., 2008) have been applied to organic synthesis because these biocatalysts are easily obtainable from markets and are easily manipulated.

An increasing number of reports dealing with the assessment of bioreduction of prochiral ketones using plants have been frequently available (Phukan et al., 2012; Matsuo et al., 2008; Sekhri et al., 2009; Yang et al., 2008; Andrade et al., 2006).

The aim of this research was to contribute to this area by choosing acetophenone as typical ketone and biochemical catalysts such as Algerian figs.

Figs are native to the Middle East and Mediterranean; one of the world's oldest trees, the fig tree can be traced back to the earliest historical documents and features prominently in the Bible and Koran. Figs are commonly known in Arabic as Attin. They belong to the genus Ficus, and their scientific name is ***Ficus carica***; Figs are fruits that grow on the Ficus tree, a member of the Mulberry family or Moraceae; They belong to the genus Ficus, and their scientific name is *Ficus carica*; figs are a culmination of many single seeded fruits and grow to a size of 3-5 centimeters. They are green while growing and turn either purple or brown once they ripen as shown in **Figure 1** and **Figure 2.**

Figure 1: Fresh green figs obtained from Tala Ali-Beni Chebana region W. Setif, Algeria

Figure 2: Fresh black figs obtained from Tala Ali-Beni Chebana region W. Setif, Algeria.

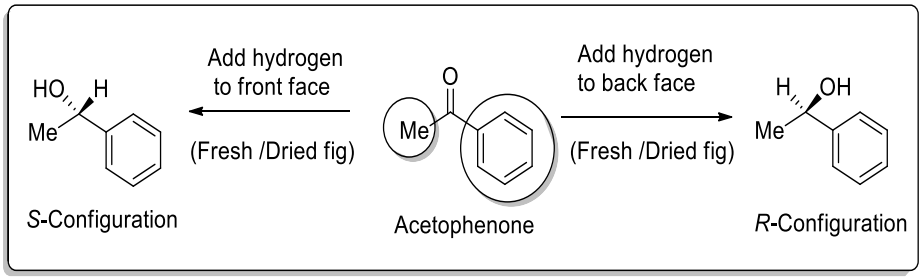
The fig tree is deciduous and can grow to the height of 7-10 meters; it grows wild in dry and sunny areas that have fresh and deep soil. It also tends to grow in rocky areas and can sustain even in less fertile soil. The fig trees can live up to 100 years. Figs are rich specially in dried state (as shown in **figure 3)**, in **vital vitamins and minerals** including Vitamins A, B1 and B2, manganese and potassium, magnesium, copper, iron, and phosphorus.

Figure 3: Dried figs obtained from Tala Ali-Beni Chebana region W. Setif, Algeria

The high level of potassium and low level of sodium give relief to people with hypertension. High calcium levels present in figs promote bone health (USDA Nutrition Database, Dried Figs). Excess oestrogen hormone in the body can lead to menopausal problems, ovarian, breast and uterine cancer, weight gain and mood swings (Suzuki et al., 2008; The World's Healthiest Foods, Figs). Figs reduce the triglyceride levels in your blood and contribute to improving your heart health. Triglycerides are fat particles in the blood that are a leading cause of heart diseases. Also, the antioxidants in figs get rid of the free radicals in the body, which block the coronary arteries and cause coronary heart disease (Vinson et al., 2005; USDA Food Composition Databases). Figs also contain phenols and omega-3 and omega-6 fatty acids that decrease the risk of heart diseases. Figs also contain beta-carotene as well as benzaldehyde (anti-cancer compound), flavonoids and a digestive enzyme called ficin. Figs contain pectin, a soluble fiber that is known to reduce cholesterol levels. The fiber in figs clears the excess cholesterol in your digestive system, and carries it to the bowels to eliminate it. Figs also contain vitamin B6 that is responsible for producing serotonin. This serotonin boosts your mood and lowers cholesterol.

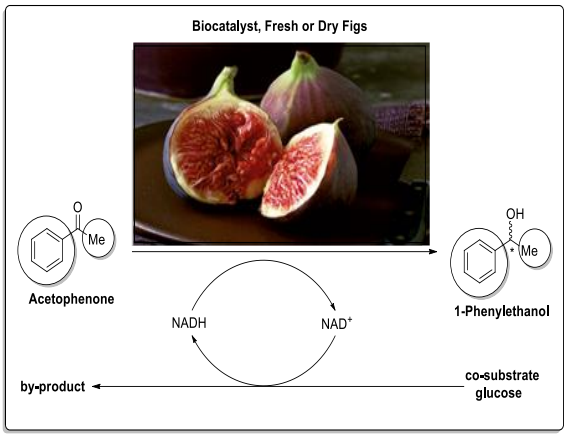
2. RESULTS AND DISCUSSION

Asymmetric transformations invariably involve the conversion of two dimensional substrate into a three dimensional product. For prochiral ketones such as acetophenone reduction shown in **Scheme 1**, addition to the back face gives alcohol with *R* configuration, while addition to the back face gives alcohol with *S* configuration. The problem of course was that most common reducing agents, such as sodium borohydride or lithium aluminium hydride, react equally readily with either faces. The most obvious solution to this problem was to use a hydride source which itself was enantiomerically pure in principal such as reagent would transfer the hydride to each face of the ketone through diastereoisomerically distinct transition state, which gives at least a fighting chance of an energy difference, and preference for addition to one face over the other.



Scheme 1: Asymmetric conversion of two dimensional substrate into a three dimensional product.

Moreover, plants are potential biocatalysts used as the alternative solution to this problem, since they are easily obtainable from markets, and are easily manipulated. Asymmetric reduction reactions of acetophenone **1a**, using the fruits of fresh/dried figs was investigated (**Scheme-2).**



Scheme 2: Asymmetric reduction reactions of acetophenone using fresh/dryFigs

3. EXPERIMENTAL

3.1. General methods

Acetophenone **1a**, was purchased from Aldrich. These chemicals were used without further purification. Thin-chromatography (TLC) was performed using precoated plates (Aluminium foil, silica gel 60 F254 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 400.15 MHz ^{1}H NMR and 100.62 MHz ^{13}C NMR spectra were run on a Bruker AC 300 NMR spectrometer. Both ^{1}H NMR and ^{13}C NMR spectra were recorded using $CDCl_3$ as internal standard; Infrared spectra were recorded using a Perkin-Elmer 783 spectrometer equipped with a PE 600 data station.

3.2. Biocatalysts

Fresh/dried black and green figs were obtained from Tala Ali-Beni Chebana region W. Setif, Algeria, and washed with water, then disinfected with ethanol. They were carefully cut into small thin pieces (approximately 1 cm long slice). A suspension of figs (50 g) in water (100 ml) was stirred in an Erlenmeyer flask at 30 ° C for 30 min., and its juice was obtained by mixing (50 g in100 ml H_2O) using an electric mixer in slightly deionized water and then, was stored at (25°C).

3.3. Standard Procedure

Typical reaction mixture consisted of (0.02 mol) appropriate ketone, acetophenone (**1a)** in dimethylformamide (DMF) (1 mL), 3% (W/V) of glucose or i-PrOH (in the case of solid ketones), 20 ml of phosphate buffer (pH = 6.5) was added to 50 g of cultured plants, fresh/ dry fig suspension in 100 mL deionized water. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at 30°C for 4 days. 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. Then chemical yield and enantioselectivity were determined. Each experiment was parallelly repeated at least three times. Then, the average value and standard deviations were given.

The products were identified by comparing their data with those of authentic samples on TLC, by IR, and ^{1}HNMR spectra (Sekhri et al., 1998; Drew et al., 1997). The presence of alcoholic group in the final product was chemically confirmed by acetyl chloride test.

3.4. Determination of optical activity of chiral products:

Optical properties of the products obtained from the prochiral were studied with the help of polarimeter Euromex Polarimeter PM. 5400 (Mitscherlich type polarimeter) using the method described in a paper reported recently (Nedjimi, Sekhri et al., 2016).

3.5. Identification of chiral alcohols 1b by optical properties and spectroscopic data

Phenylethanol 1b:

Using Fresh figs: *(R)*-**(1b)** was obtained in (78% yield), $[\alpha]_D^{20}$ = +38 (*c* 5, MeOH); enantiomeric excess (ee= 76%; 88% *R*). The absolute configuration was estimated by analogy with {Lit., (Aldrich, 1995/1996) $[\alpha]_D^{20}$ =+84 (*c* 5, MeOH) for *R*-isomer}.

Using dried figs:

(R)-**(1b)** was obtained in (85% yield), $[\alpha]_D^{20}$ = +36 (*c* 5, MeOH); enantiomeric excess (ee= 80%; 90%*R*). The absolute configuration was estimated by analogy with {Lit., (Aldrich, 1995/1996) $[\alpha]_D^{20}$ =+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 (Sekhri et al., 1998; Drew et al., 1997).

vmax (KBr Disk, cm^{-1}): (OH). 3340-3060; ^{1}H ($CDCl_3$; 400,15 MHz): δ (ppm): 1.48 (3H, d, CH_3CHOH-), 4.80 (1H, q,-CHOH) 3.99 (1H, br.s, OH), 7.25-7.36 (5H, m, Ar-H)**;** ^{13}C ($CDCl_3$; 100,62 MHz): δ (ppm): 22.81(CH_3CHOH), 69.9 (-CHOH), 127.1 (-CH, Ar), 127.6 (-CH, Ar), 128.9 (-CH, Ar), 146.1 (C, Ar).

4. CONCLUSION

The bioreduction of acetophenone with **fresh/dried figs** could be effectively reduced to the corresponding chiral alcohol by the applied plant tissue. The reason was that aromatic ketones such as acetophenone were more acceptable to plant cells. Moreover, only *R*- form configuration could be obtained through these asymmetric reduction reactions. This provided a new route to produce chiral alcohols, as the platform chemicals for enantiomerically pure pharmaceuticals, through asymmetric reduction of the corresponding prochiral ketones.

Among various co-substrates, glucose found to be the best for regeneration of co-factors and **fresh/dry figs** was chosen as the biocatalysts. The acetophenone derivatives could be reduced to the corresponding chiral alcohols with attractive enantioselectivity by fresh fruits of this plant. Dried figs gave

more or less the same selectivity (enantiomeric exceess) since the enzymes responsible for selectivity were still present, and were not destroyed during the drying process.

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