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By

Ritu Saharan and Jyoti Joshi

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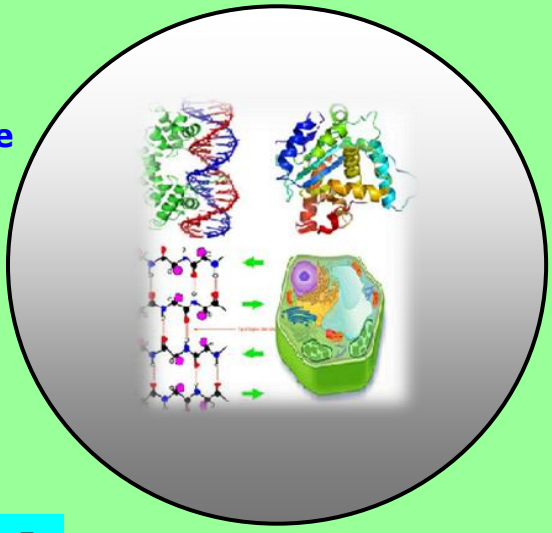

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Dr. Ritu Saharan
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RESEARCH PAPER

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Synthesis, Characterization and Antibacterial Studies of 4-Hydroxy-3-Methoxy-Benzyl Alcohol and 1-(4-Hydroxy-3-Methoxyphenyl) Ethanol

Ritu Saharan and *Jyoti Joshi

Department of Chemistry, University of Rajasthan, Jaipur, 302004, India
***Department of Chemistry, MNIT Jaipur, 302017, India**

ABSTRACT

The synthesis and characterization of aromatic alcohols such as 4-Hydroxy-3-methoxy-benzyl alcohol and 1-(4-Hydroxy-3-methoxyphenyl) ethanol using microbial transformation (via whole cells of Baker's Yeast in their free as well as immobilized form in mixtures of glycerol and water) are reported. The products obtained were purified and then characterized by spectroscopic techniques viz. IR, ^{1}H NMR and Mass spectra. Both the aromatic alcohols are found to be potential bioactive material against the pathogenic bacteria.

Keywords: Microbial catalyst, Baker's yeast, Immobilization, Aromatic alcohol and Antibacterial screening.

INTRODUCTION

Microbial transformation employing enzymes has advantages such as their ability to carry out a wide range of organic reactions at higher reaction rates over conventional chemical reactions in organic synthesis Loughlin et al. (2000) and Zarevucka et al. (2003). Microbial catalyst assisted reactions are economical viable, environmental friendly, performed at mild temperature and neutral pH, possess chemo, regio and stereo selectivity Petersen et al. (1999). By using biocatalysts drug analogs can be manufactured and evaluated for their pharmacological activity, toxicity or pharmacokinetics with respect to starting compounds. According to Carballeira et al. (2009) Microbial transformations have also served as a model for the evaluation of the drug metabolism in mammalian organisms. Khor et al. (2011) and Nakamura et al. (2003) suggested, in the field of biotechnology, the use of enzymes and

microorganisms can avoid not only unwanted side reactions but also provide less hazardous and less toxic products as compared with conventional chemical catalysts. Wolfson et al.(2008) reported that among various kinds of Microbial transformation, reduction of carbonyl compounds using Baker's Yeast for producing corresponding alcohols is a convenient and useful synthetic route due to its eco friendly nature, low cost and easier handling.

For Baker's Yeast mediated reductions, water is the foremost choice of solvent but it has numerous disadvantages such as low solubility of the organic substrate, undesired side reactions and difficulties involved in isolation of the product. Therefore the reduction of various prochiral ketones has also been studied in different organic solvents such as hexane, toluene, and benzene but under these conditions cells are damaged and has also severe environmental impact, Wolfson et al. (2007).

4-hydroxy-3-methoxy-benzyl alcohol (Vanillyl alcohol), is used for production of flavoring ingredients, biologically active moleculesandin chemical communication system of insect species, such as African sugar-cane borer moth and the Leaf footed pine seed bug. Hsu et al. (2009) suggested that, to cure Parkinson's disease, Vanillyl alcohol is used. Akkerman et al. (1997) reported Hydroxyl-methoxy-sulfonic acid-benzyl alcohol (HMSBA) that is potassium salt of Vanillyl alcohol used for treating cancerous tumors.1-(4-hydroxy-3-methoxyphenyl) ethanol (Apocynol) is used as a simple model for lignin. Aromatic alcohols were proved to have biological activity against bacteria.

MATERIAL AND METHODS

All chemicals used in the present investigation were of analytical grade and their purity has been further checked by single spot TLC. Double distilled water was used for preparing all the solutions used in experiments. ^{1}H NMR spectra were recorded using Joel (Japan) 300MHZ spectrophotometer. FT-IR spectra were recorded from Nicolet (USA) FT-IR spectrophotometer. Mass spectral analysis has been done in Central Drug Research Institute (CDRI), Lucknow.

Reduction using microbial catalyst (Baker's Yeast)

In microbial catalyzed reduction, immobilization of Baker's Yeast in 5% polyacrylamide gel has been carried out by preparing following solutions.

Solution A: - 10g Acryl amide and 2.5g N, N-methylene bis acrylamide in 100ml doubled distilled water.

Solution B: - 5.98g Trihydroxy methyl amino methane, 0.46ml N, N, N', N"-tetramethyl ethylenediamine and 48ml 1N Hcl solution to 100ml solution.

Solution C: - 560mg Ammonium per sulphate in 100ml doubled distilled water.

Solution D: - 34.2 g Sucrose in 100ml doubled distilled water.

These solutions were then mixed in the following sequence:-

Solution A (10ml) + Solution B (5ml) + Solution D (20ml) + Baker's Yeast (5g) and Solution C (5ml)

The resulting solution was then deareated and allowed to polymerize for nearly 1hr. The resulting gel was cut into small pieces.

Asymmetric Reduction

In a 500 ml flat bottom flask, a mixture of water and glycerol (50:50), fresh Baker's Yeast (free or immobilized) (10 g), sucrose (10g) was placed and the suspension was stirred for 30 minutes.

Chosen carbonyl compound (2mM) dissolved in minimum amount of absolute alcohol was then poured into the suspension. The resulting mixture was magnetically stirred for appropriate time (Table 1). After completion of the reaction, the product was filtered using celite (filter aid powder), extraction was done with diethyl ether (30ml) and the procedure was repeated three times. The ether was first evaporated from ether extract and then dried over calcium chloride to yield the product which was then characterized by boiling point determination and spectral studies viz. IR, ^{1}H NMR and Mass spectral analysis (Table-1 andTable-2).

Antibacterial screening

The in vitro growth inhibitory actions of the synthesized4-Hydroxy-3-methoxybenzyl alcohol and 1-(4-Hydroxy-3-methoxyphenyl) ethanol were tested by using the Disc Diffusion method against the bacteria *E. coli, S. aureus*, *P. aeruginosa* and *E. faecalis*. The bacteria were cultured in nutrient agar medium in Petri plates and used as inoculums for the study. A measured quantity of the test compounds such as4-Hydroxy-3-methoxy-benzyl alcohol and 1-(4-Hydroxy-3-methoxyphenyl) ethanol were dissolved in methanol to get final concentrations of 250 ppm and soaked in filter paper discs of 5mm diameter. These discs were placed on the previously seeded plates and incubated at 35^{0}C. The diameter (in millimeter) of inhibitory zone around each disc was measured after 24 hours. Filter paper disc treated with methanol served as control and Streptomycin (2.5 mg) used as reference drugs.

RESULTS AND DISCUSSION

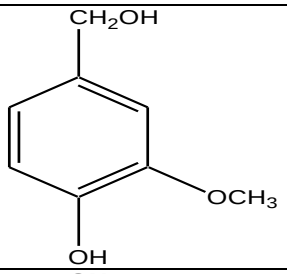
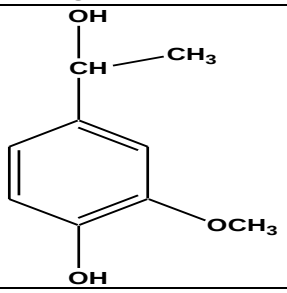
Reduction using microbial catalyst (Baker's Yeast)

The whole cells of Baker's Yeast as microbial catalysts in asymmetric reduction of carbonyl compounds, involve two enzyme systems. One is the enzyme catalyzing the asymmetric reduction and other is the cofactor regeneration system by which NADH is regenerated through the oxidation of energy source such as carbohydrates.

An extra cellular invertase (β-D-fructosidase), is found in *Saccharomyces cerevisiae* cells which hydrolyzes sucrose into glucose and fructose. These glucose and fructose are transported into the cell by hexose transporters and metabolized through glycolysis. Addition of sucrose to the reaction mixture increases the bioreduction. It is due to enhanced regeneration of the co-factor in Baker's Yeast in the presence of glucose that uses as electron donor.

Though from the viability and activity point of view, water is the appropriate and natural solvent for microbial catalysis. Glycerol is an alternative green solvent. It has the advantage such as solubility of substrate and easier product separation. Therefore while carrying out microbial reduction (using either free or immobilized whole cells), asymmetric reduction in a mixture of water and glycerol i.e. 50:50, has advantages of both the solvents. The reduction carried out using whole cells of immobilized Baker's yeast gave high yield as compared to free whole cells due to enhanced operational stability of free Baker's Yeast, easier isolation of the products and repeated use of biocatalyst.

Table. 1 Physical data from free as well as immobilized Baker's Yeast mediated Reduction.

Product		Reaction time (in hours)		Melting point (^{0}C)	FBY Yield (%)	ImBY Yield (%)
Name	**Structure**	**FBY**	**ImBY**			
4-Hydroxy-3-methoxy-benzyl alcohol		72	96	113	78	83
1-(4-Hydroxy-3-methoxyphenyl) ethanol		72	96	101	75	79

FBY= Free Baker's Yeast
ImBY= Immobilized Baker's Yeast

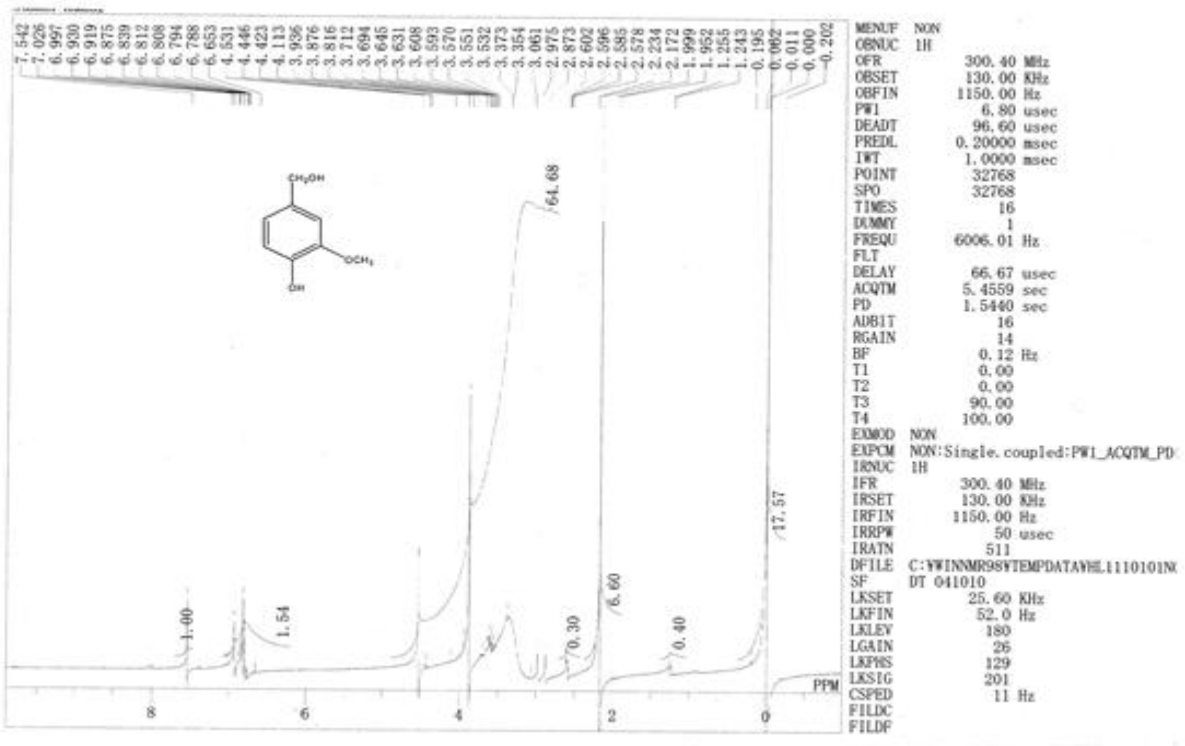


Fig. 1. ^{1}HNMR spectrum of 4-Hydroxy-3-methoxy benzyl ethanol.

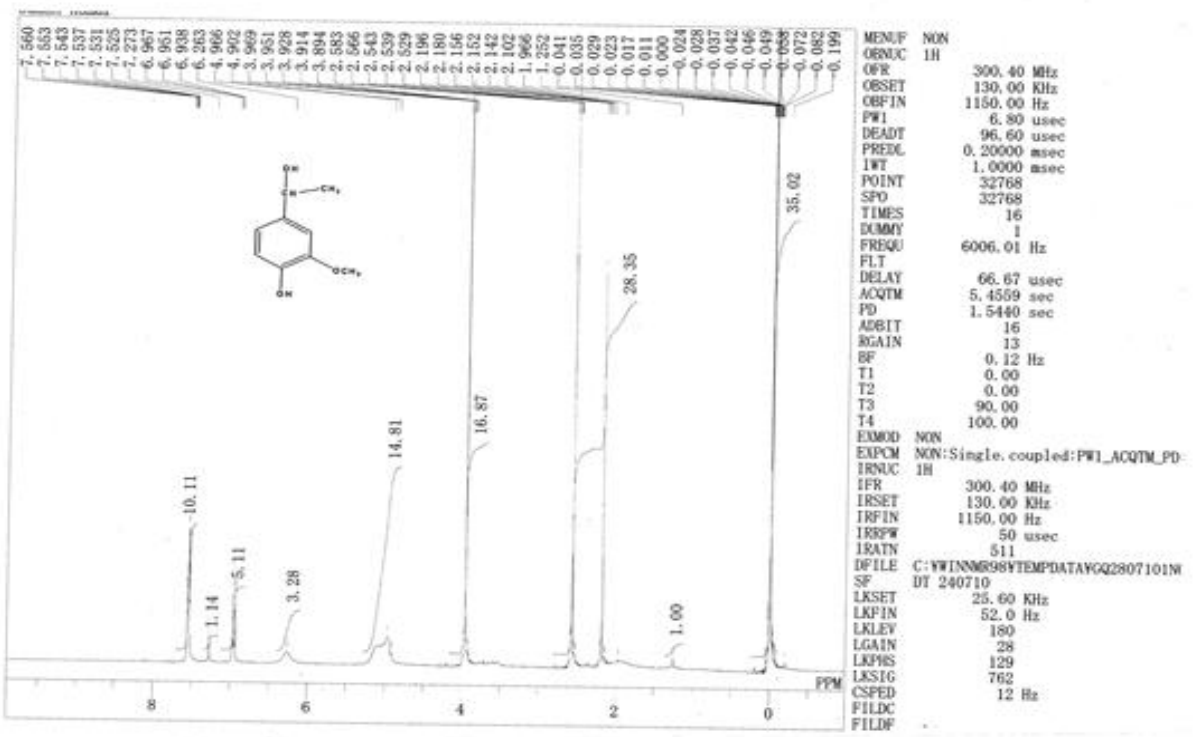
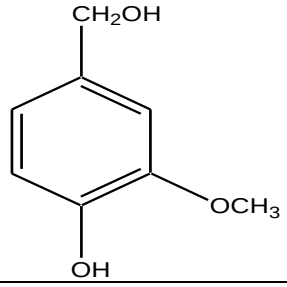
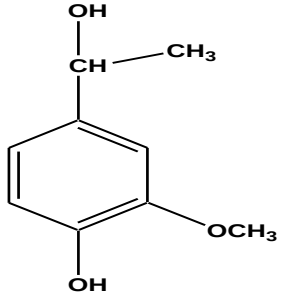
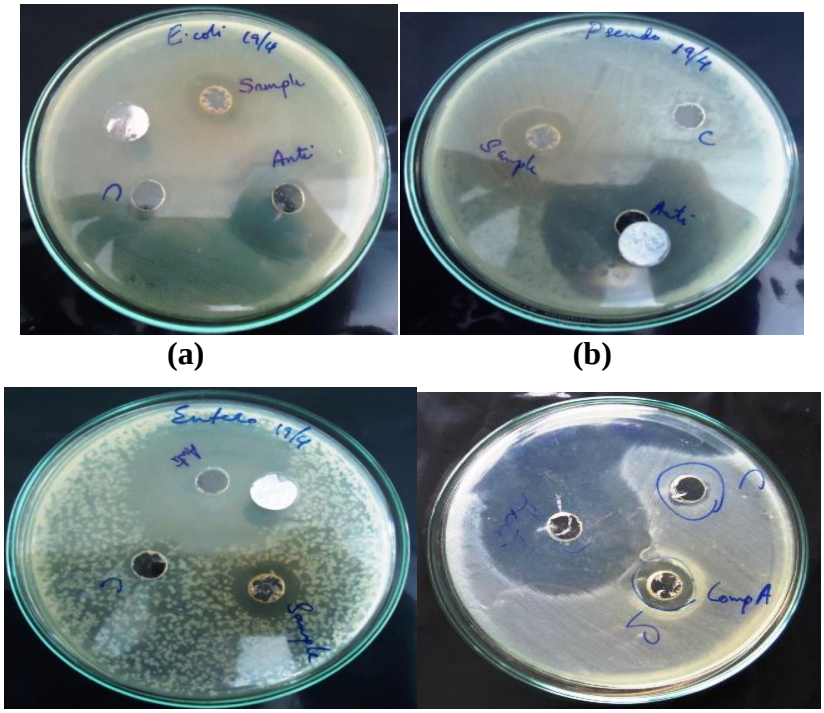


Fig. 2. ^{1}HNMR spectrum of 1-(4-Hydroxy-3-methoxy phenyl) ethanol.

Table. 2 Spectral data from free as well as immobilized Baker's Yeast mediated Reduction.

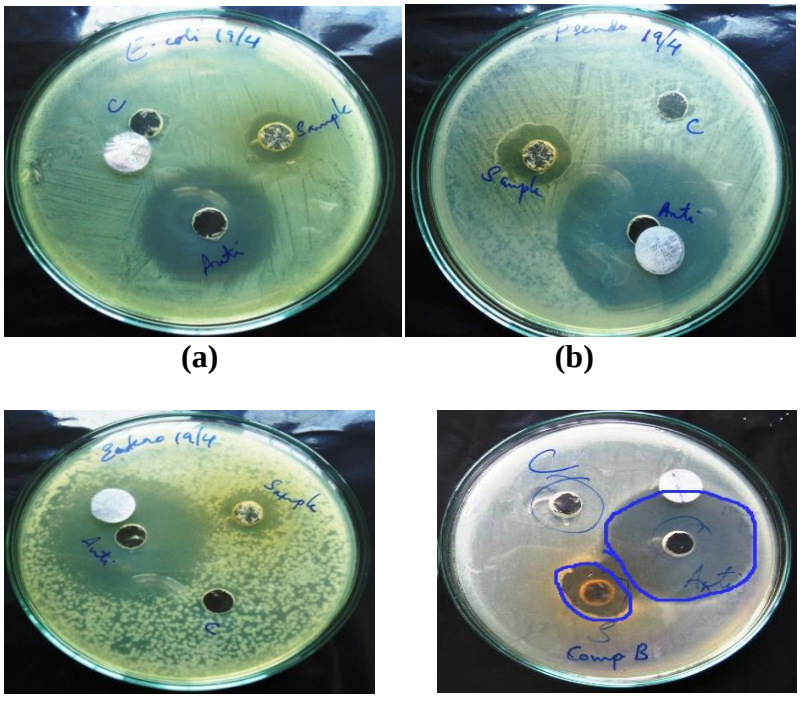
Product		**IR Data (cm^{-1})**	**^{1}H NMR Data (δ)**	**Mass Data m/z (M^{+})**
Name	**Structure**			
4-Hydroxy-3-methoxy-benzyl alcohol		3360(OH), 3020(Ar C-H str), 2965(CH-str), 1600,1525,1470(C=C ring str), 1200&1380(C-O strPh), 1050 (C-O str primary alcohol)	2.1(O**H**), 3.7(-OC**H$_3$**), 4.5(-CH-**OH**), 6.8(ortho-C**H**), 6.7(meta-C**H**),	154.08
1-(4-Hydroxy-3-methoxy phenyl) ethanol		3350(OH), 3000(Ar C-H str), 2970(CH-str), 1605,1520,1470(C=C ring str), 1200&1370(C-O str Ph), 1100&1295 (C-O str Sec.alcohol)	2.0(O**H**), 3.7(-OC**H$_3$**), 5.0(-CH-**OH**), 6.1(ortho-C**H**), 6.5(meta-C**H**),	169 (M+1)



(a) **(b)**

(c) **(d)**

Figure 3. Antibacterial activity pattern of 4-Hydroxy-3-methoxybenzylalcohol against (a) *Escherichia coli*(b) *Pseudomonas aeruginosa* (c) *Enterococcus faecalis* (d) *Staphylococcus aureus*



(a) **(b)**

(c) **(d**

Figure 4. Antibacterial activity pattern of 1-(4-Hydroxy-3-methoxyphenyl) ethanol against (a) *Escherichia coli* (b) *Pseudomonas aeruginosa* (c) *Enterococcus faecalis* (d) *Staphylococcus aureus.*

Antibacterial screening

The all synthesized aromatic alcohols such as 1-(4-hydroxy-3-methoxyphenyl) ethanol and 4-hydroxy-3-methoxy-benzyl alcohol have been tested for the *in vitro* growth inhibitory activity against the bacteria *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeuruginosa* (ATCC 27853), *Enterococcus faecalis* (MTCC 439), by using the well diffusion method as shown in figures (3-4).

From the results (Table-3), it is concluded that both the tested compounds exhibit moderate antibacterial activity against all species of bacteria used in this study. Antibacterial effect of these compounds on gram positive and gram negative bacteria may because by perturbation of the lipid function of microorganism, resulting into change of membrane permeability and out flow of intracellular materials or penetration of chemicals into the cell to disintegrate the cell interiors.

Table 3. Antibacterial screening data of 4-Hydroxy-3-methoxybenzylalcohol and 1-(4-Hydroxy-3-methoxyphenyl) ethanol.

S. No.	Product Name	Diameter of inhibition zone (mm)			
		***Escherichia coli* (gram negative)**	***Staphylococcus aureus* (gram negative)**	***Pseudomonas aeruginosa* (gram positive)**	***Enterococcus faecalis* (gram positive)**
1.	4-Hydroxy-3-methoxy benzyl alcohol	25mm	22mm	23mm	19mm
2.	1-(4-Hydroxy-3-methoxyphenyl) ethanol	20mm	16mm	21mm	18mm
	Streptomycin (Reference)	35mm	37mm	34mm	33mm

CONCLUSION

Aromatic alcohols have been synthesized using microbial transformation using Baker's Yeast (in a mixture of glycerol and water) and characterized on the basis of analytical and spectral data. Microbial transformation provides an alternative opportunity to synthesize pharmaceutically important chiral compounds useful in the manufacture of drugs. This synthetic approach is a green methodology over conventional chemical methods as Microbial transformation is effective, safe, cost-effective, ecofriendly, easy to handle. In addition the synthesized 4-hydroxy-3-methoxy-benzyl alcohol and 1-(4-hydroxy-3-methoxyphenyl) ethanol are biological active material against the pathogenic bacteria.

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Corresponding author: Dr. Ritu Saharan, Department of Chemistry, University of Rajasthan, Jaipur, 302004, India
Email: ritu.saharan84@gmail.com