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Green Chemical Synthesis, Characterization and Antibacterial Studies of 2-Phenylethanol.

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ABSTRACT

The green chemical synthesis and characterization of 2-phenyl ethanol using Biocatalytic reduction (in mixtures of glycerol and water via whole cells of Baker's Yeast in their free as well as immobilized form) and electrochemical technique are reported. The electrochemical behavior of 2-phenyl ethanol was analyzed through cyclic voltammetric studies at glassy carbon electrode (GCE) followed by constant current electrolysis. Effect of scan rate and pH on the reduction reaction peak has been calculated. The kinetic parameters were also calculated and the process was found to be diffusion controlled. The products obtained were purified and then characterized by spectroscopic techniques. 2-phenyl ethanol has the optimum characteristics required for antibacterial activity and it is found to be potential bioactive material against the pathogenic bacteria.

Keywords: 2-phenyl ethanol, Biocatalytic reduction, Baker's Yeast, cyclic voltammetry, Constant current electrolysis.

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INTRODUCTION

Incorporation of green methodologies in chemical synthesis becomes a novel and efficient routes to provide relevant chiral building blocks for fine chemicals and pharmaceuticals. Biocatalytic reactions are economical viable, environmental friendly, performed at mild temperature & neutral pH, possess chemo, regio and stereoselectivity [1]. By using biocatalysts, drug analogs can be manufactured and evaluated for their pharmacological activity, toxicity or pharmacokinetics with respect to starting compounds. Biotransformations have also served as a model for the evaluation of the drug metabolism in mammalian organisms [2]. 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 [3-4].

Among various kinds of biotransformation, 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 [5-6].

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 [7].

Electrochemical synthesis is a very simple and useful synthetic method [8]. The most important advantage of the electrochemical procedure is that purification of the final product become very easy and novel products can be obtained because these reactions can be performed in absence of oxidizing or reducing reagents. The electrochemical synthesis is an alternative to provide not only pollution free but also economically viable approach for the synthesis of the organic compounds [9] because it reduces the use of at least one hazardous chemical reagent. These reactions reduce the possibility of corrosion, accidental release and also carried out in a low-temperature environment [10]. The kinetics and mechanisms of any chemical reaction can be investigated via Electrochemical techniques therefore electro organic syntheses offer alternative green synthetic approach [11].

2-phenylethanol having a rose like odour is utilized in cosmetics and perfume as fragrance chemical. It is stable to alkali therefore preferred in soap industry. It is also used in candy, soft drinks and cookies as flavour substances. Its ester, Phenylethylacetate, is a valuable flavour and fragrance compound [12].

In light of the above applications, the present work relates to the reduction of phenylacetaldehyde to 2-phenylethanol employing Biocatalysis using Baker's Yeast (in a mixture of glycerol and water) and Electrochemical reduction using cyclic voltammetry and constant current electrolysis & reports the results of the antibacterial evaluation undertaken.

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MATERIALS 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 Biocatalyst (Baker's Yeast)

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

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

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

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

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

These solutions were then mixed in the following sequence:-

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

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 (10 g) was placed and the suspension was stirred for 30 minutes. phenylacetaldehyde (2 mM) 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-2).

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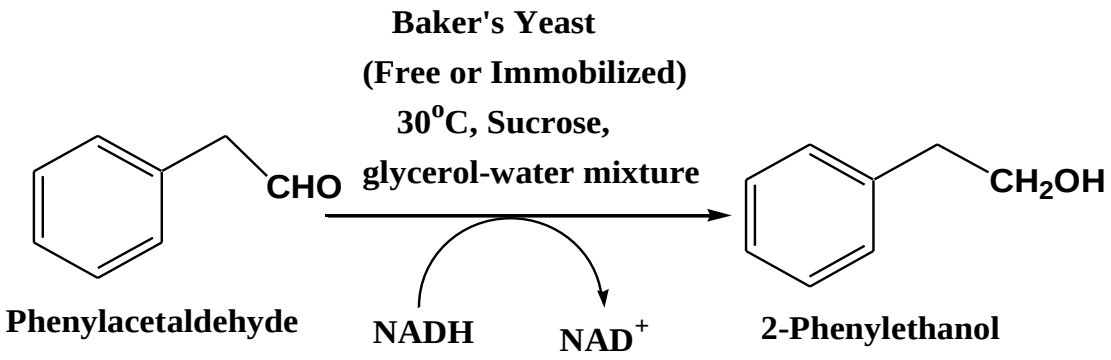

Scheme: 1 Baker's Yeast mediated Reduction of phenylacetaldehyde

Reduction using Electrochemical technique

The Basic Electrochemistry System model ECDA-001 (completely computer controlled) was used for recording cyclic voltammograms of phenylacetaldehyde at different pH and scan rates in aqueous methanol using potassium chloride as supporting electrolyte at glassy carbon electrode. Cyclic voltammetric studies were carried out using a glassy carbon working electrode (A = 0.1 mm^2), Ag/AgCl reference electrode and a platinum auxiliary electrode. All the measurements were carried out at room temperature. The working electrode was polished intensively with aluminium oxide (0.4 μ) on a polishing cloth and degreased in methanol prior to each electrochemical measurement. The solutions were purged with purified clean dry nitrogen for 5 min prior to the experiments in order to remove dissolved oxygen from the media and blank cyclic voltammograms were recorded. Solution of 1mM of phenylacetaldehyde was added to blank solution. This has again been deoxygenated then initial potential, switching potential, scan rate and current sensitivity were provided for recording the cyclic voltammograms of phenylacetaldehyde.

Constant Current Electrolysis

Phenylacetaldehyde was subjected to constant current electrolysis at constant current (1.0 amp) for 8 hrs in aqueous methanol. Galvanostat supplied by OMEGA type ICVD 60/2 was used to perform the experiment. A Remi hot plate cum magnetic stirrer (2 M LH model) was used to stir the solution throughout the electrolysis.

A two compartment H- shaped glass cell provided with a fritz glass disc (G-4) was used for electrolysis. Rectangular plates of stainless steel (SS-316) each of size (4 cm × 6 cm) was used as cathode as well as anode. The Britton Robinson buffer of appropriate pH and the supporting electrolyte (CH_3COONa) was filled in both the limbs of H-shaped glass cell. phenylacetaldehyde was dissolved in minimum amount of methanol and placed in the cathodic compartment and electrolyzed at constant current (1.0 amp). After the completion of reaction, extraction was done with diethyl ether (30 ml) 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, ^1H NMR and Mass spectral analysis (Table-3 & Table-4).

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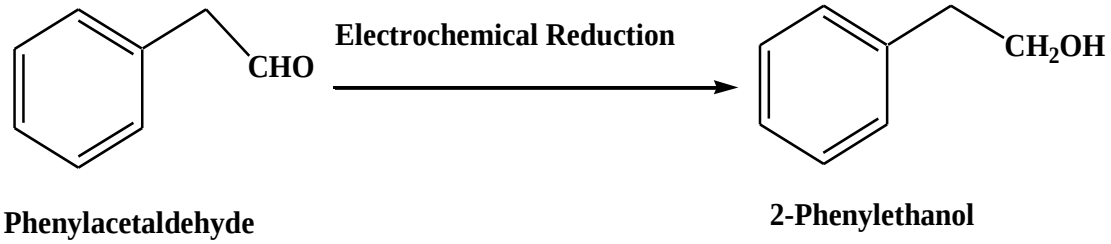

Phenylacetaldehyde 2-Phenylethanol

Scheme: 2 Electrochemical Reduction of phenylacetaldehyde

Antibacterial Activity

The synthesized 2-phenyl ethanol was tested for the in vitro growth inhibitory action against the bacteria *E. coli, S. aureus*, *P. aeruginosa* and *E. faecalis* by using the Disc Diffusion method. The bacteria were cultured in nutrient agar medium in Petri plates and used as inoculums for the study. Measured quantities of the 2-phenyl ethanol was dissolved in methanol to get final concentrations of 250 ppm and soaked in filter paper discs of 5 mm diameter. These discs were placed on the previously seeded plates and incubated at 35^0C. 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 Biocatalyst (Baker's Yeast)

Asymmetric reduction of carbonyl compounds using whole cells of Baker's Yeast as biocatalysts involve two enzyme systems. One of them is the enzyme catalyzing the asymmetric reduction and other is the cofactor regeneration system, which regenerate NADH through the oxidation of the energy source such as carbohydrates. *Saccharomyces cerevisiae* cells have an extra cellular invertase (β-D-fructosidase), which hydrolyzes sucrose into glucose and fructose, which 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.

Although water is the most suitable, natural and natural solvent for biocatalysis from the viability and activity point of view, an alternative green solvent is glycerol. It has the advantage with respect to substrate solubility and product separation. Therefore asymmetric reduction in a mixture of water and glycerol has advantages of both the solvents while carrying out the reduction using either free or immobilized whole cells. 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, isolation of the products and repeated used.

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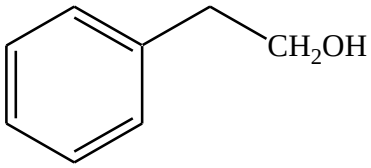
Product		Reaction time (hrs)	Boiling point (^{0}C)	FBY Yield (%)	ImBY Yield (%)
Name	Structure				
2-phenyl ethanol		72	218	81	83

Table: 1 Physical data from Free as well as Immobilized Baker's Yeast mediated Reduction

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

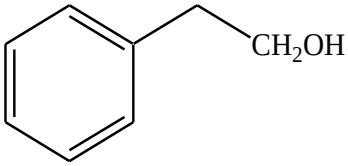
Product		IR Data (cm^{-1})	^{1}H NMR Data (δ)	Mass Data m/z (M^+)
Name	Structure			
2-phenyl ethanol		3340 (OH), 3040 (Ar C-H Str), 2880-2940 (C-H str), 1500,1460 (C=C ring str), 1050 (C-O str. Primary alcohol), 680-760 (strong Aromatic absorption as C-H out of plane bend. Vib.)	2.1; OH (s), 3.8; 2H (s), 7.2; Ar-5H (m)	123.01 (M+1)

Table: 2 Spectral data from Free as well as Immobilized Baker's Yeast mediated Reduction

Reduction using Electrochemical technique

In the cyclic voltammograms of phenylacetaldehyde at pH 5.0, pH 7.0 and pH 9.0 single irreversible cathodic peak was observed due to the reduction of >C=O moiety to the corresponding alcohol yielding 2-phenyl ethanol as final product. Kinetic Parameters evaluated from cyclic voltammograms are given in Table 5.

Effect of scan rate

The effect of scan rate on cathodic peak potential (E_{pc}) was studied. Fig. 1 shows the effect of scan rate on cathodic peak potential (E_{pc}). On increasing the scan rate, the cathodic peak potential (E_{pc}) is shifted towards more negative potentials indicating an irreversible electron transfer process. The dependence of the voltammetric peak current (I_{pc}) of the wave on the square root of scan rate ($v^{1/2}$) is linear with correlation coefficients close to unity at all the pH (Graphical representation in fig. 3) . Under these conditions the current process was diffusion controlled. Thus phenylacetaldehyde was reduced electrochemically in a diffusion-controlled irreversible cyclic voltammetry wave.

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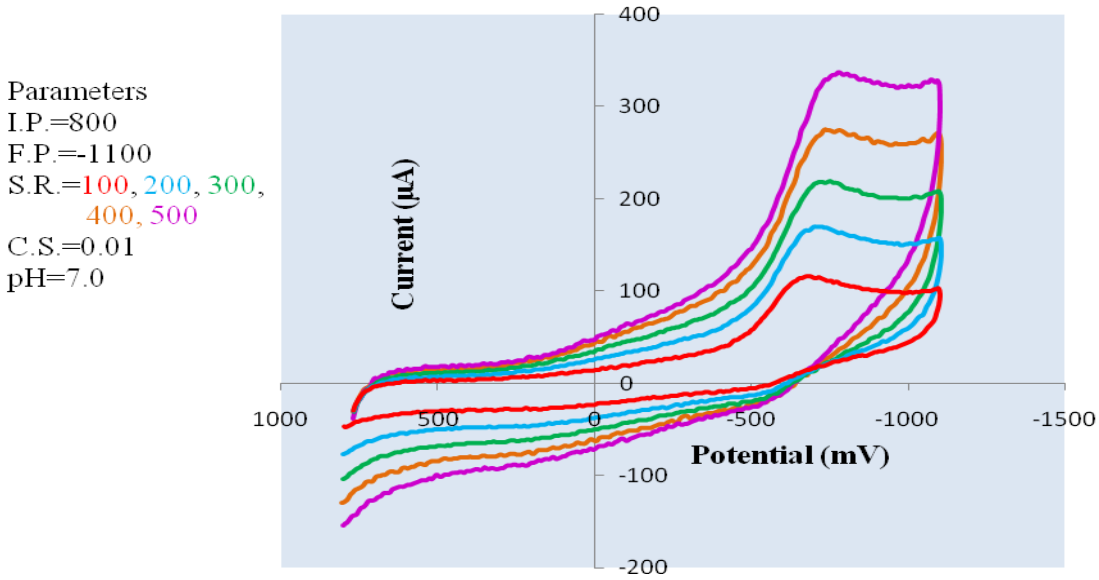

Fig. 1 Cyclic voltammograms of phenylacetaldehyde at various scan rates at pH 9.0 where I. P. =Initial potential, F. P. =Final potential, S.R. = Scan rate, C.S. =Current sensitivity

Effect of pH

Effect of pH can be explained on the basis of cyclic voltammograms obtained at different pH. Cyclic voltammograms show that the reduction can be best carried out in basic medium because a very well defined peak is observed in basic medium as compared to acidic and neutral medium where peak remains absent or observed very weakly respectively.

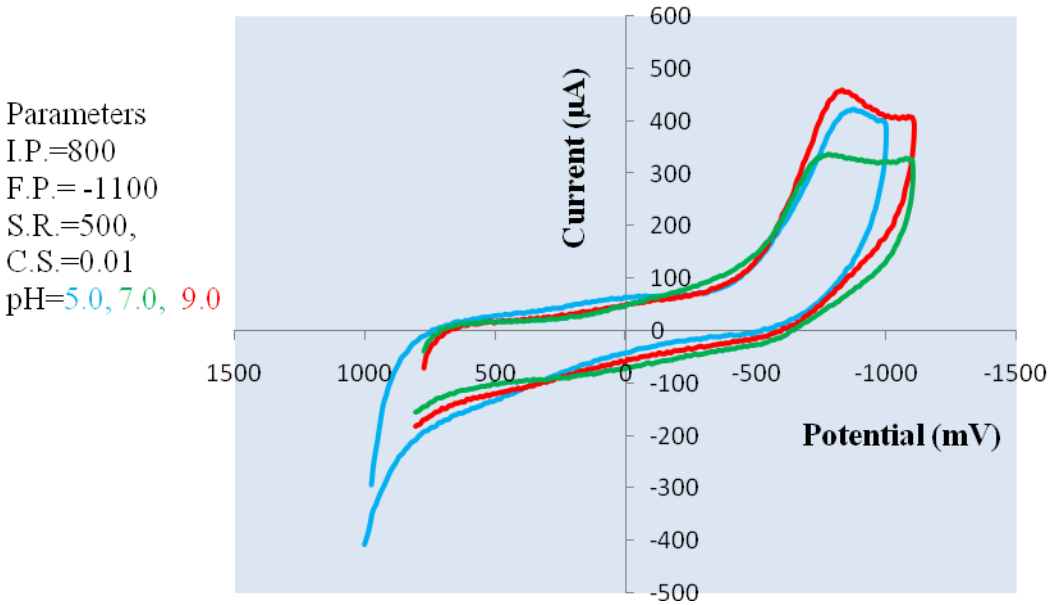

Fig. 2 Cyclic voltammograms of phenylacetaldehyde at different pH where I.P. =Initial potential, F.P=Final potential, S.R.=Scan rate, C.S.=Current sensitivity

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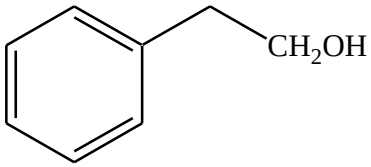
Product		Reaction time (hrs)	Boiling point (^{0}C)	Yield (%)
Name	Structure			
2-phenyl ethanol		8	218	81

Table: 3 Physical data from Electrochemical Reduction

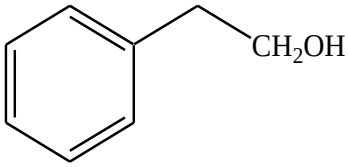
Product		IR Data (cm^{-1})	^{1}H NMR Data (δ)	Mass Data m/z (M^+)
Name	Structure			
2-phenyl ethanol		3350 (OH), 3040 (Ar C-H Str), 2895 (C-H str), 1525, 1465 (C=C ring str), 1050 (C-O str. Primary alcohol), 680-760 (strong Aromatic absorption as C-H out of plane bend. Vib.)	2.1 (OH), 3.8 (CH_2-OH), 7.2 (Ar-CH)	123.01 (M+1)

Table: 4 Spectral data from Electrochemical Reduction

S. No.	Compound name	Scan rate v (mV/s)	Cathodic peak potential E_{pc}(mV)	Cathodic peak current I_{pc}(μA)	peak current / square root of scan rate (Ip/√v)
1.	phenylacetaldehyde	100	- 649	123	12.3
		200	- 658	178	12.58
		300	- 681	225	12.99
		400	- 704	254	12.7
		500	- 752	287	12.85

Table: 5 Voltammetric data evaluated from cyclic voltammograms of phenylacetaldehyde

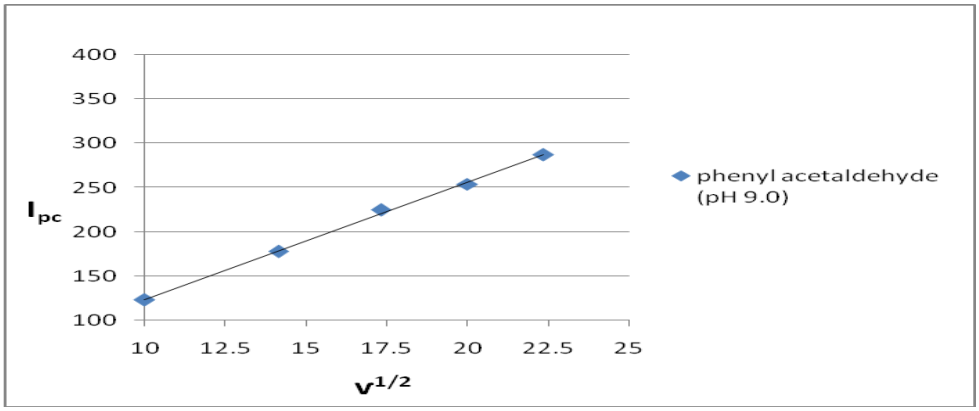


Fig. 3 Graphical representation of relation between cathodic peak current (i_{pc}) and $v^{1/2}$ for phenyl acetaldehyde at pH 9.0

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Antibacterial Activity

The synthesized 2-phenyl ethanol was examined for the invitro growth inhibitory action against *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa* by using the Disc Diffusion method as shown in figures 4 (a-d). From the results as shown in Table 6, it is concluded that 2-phenyl ethanol exhibit wide spectrum of activity as it is highly active against all bacterial cultures.

From the observation it can be inferred that 2-phenyl ethanol has the optimum characteristics required for antibacterial activity probably because of its activity to interact with cell membrane by either changing the proton motive force or the pH gradient or electrical potential or a combination of them.

An important parameter for antibacterial activity of a material is hydrophobicity as it is directly linked with membrane permeability. According to Hunt the potency of aliphatic alcohols is related to hydrophobic interaction between alkyl chains of alcohols and lipid part of membrane [13]. Similarly hydrophobic interaction might be found between alkyl chain of phenols and lipid content of the bacterial membranes. As a result of this hydrophobic interaction the membrane permeability of bacterial cell is lost which causes death of bacterial cell [14].

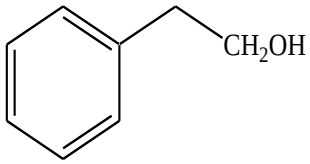
Aromatic alcohol	Structure	Diameter of inhibition zone (mm)			
		E. coli (gram negative)	*S. aureus* (gram positive)	*P. aeruginosa* (gram negative)	*E. faecalis* (gram positive)
2-phenyl ethanol		24 mm	27 mm	22 mm	21mm

Table: 6 Antibacterial Screening data of 2-phenyl ethanol

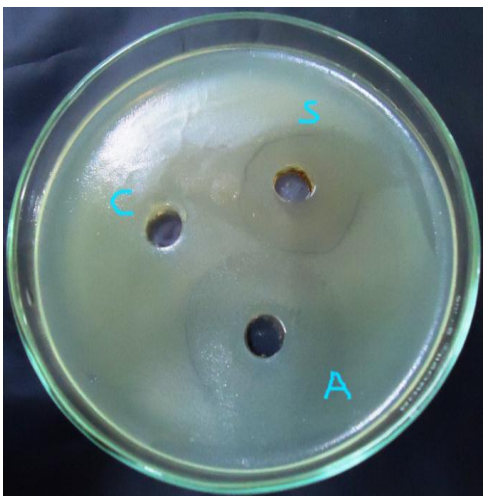


Fig: 4 (a) Antibacterial activity against *Pseudomonas aeruginosa*

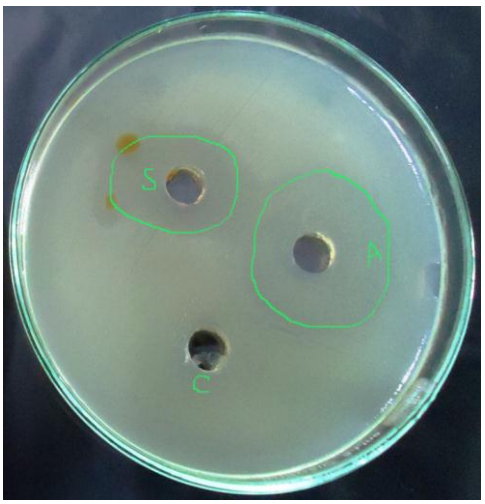


Fig: 4 (b) Antibacterial activity against *Enterococcus faecalis*

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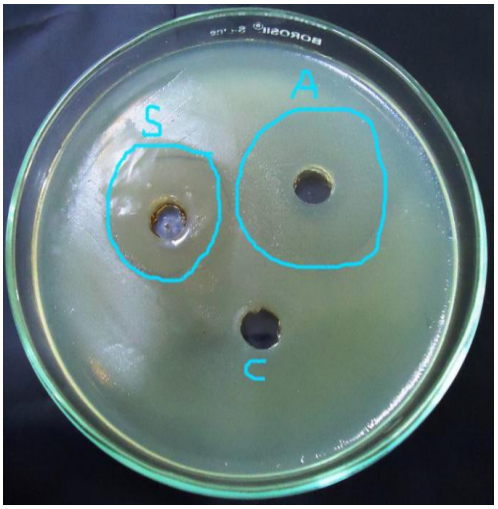

Fig: 4 (c) Antibacterial activity against *Escherichia coli*

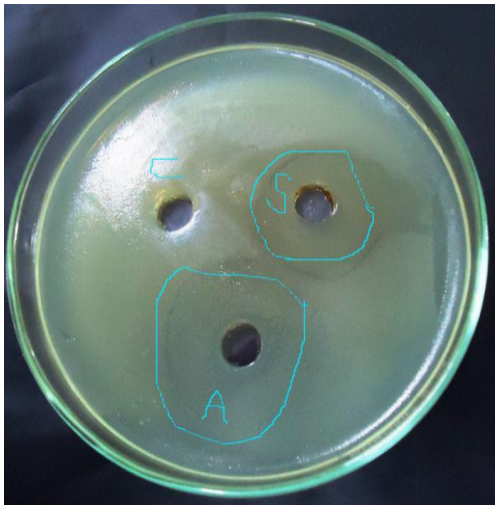

Fig: 4 (d) Antibacterial activity against *Staphylococcus aureus*

CONCLUSION

2-phenyl ethanol has been synthesized using Biocatalytic and Electrochemical techniques and characterized on the basis of analytical and spectral data. Biocatalytic reduction provides an alternative opportunity to synthesize pharmaceutically important chiral compounds useful in the manufacture of drugs. These two synthetic approaches follow green methodology over conventional chemical methods in terms of effectiveness, safety, economical, ecofriendly, easy to handle and provide new and improved synthetic routes to many valuable compounds in fields of pharmaceutical, flavor and perfume industry. In addition, the synthesized 2-phenyl ethanol is found to be potential biocidal material against the pathogenic bacteria.

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