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SYNTHESIS, CHARACTERIZATION AND BIOCIDAL STUDIES OF SOME AROMATIC ALCOHOLS

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ABSTRACT : The synthesis and characterization of aromatic alcohols such as 1-(4-bromo phenyl) ethanol, 1-(4-Hydroxy-3-methoxyphenyl) ethanol, (4-Hydroxy-3-methoxy-benzyl alcohol) employing biotransformation (using whole cells of Baker's Yeast in their free as well as immobilized form in mixtures of glycerol and water) and Electrochemical technique are reported. The electrochemical behavior of 4-bromoacetophenone, 4-Hydroxy-3-methoxyacetophenone, and 4-Hydroxy-3-methoxybenzaldehyde was analyzed using cyclic voltammetry at glassy carbon electrode (GCE) and constant current electrolysis. Effect of scan rate and pH on the reduction peaks has been calculated. The kinetic parameters were also calculated and the process was found to be diffusion controlled. The products obtained were purified & then characterized by spectroscopic techniques. All the compounds have been tested in vitro against a number of microorganisms in order to assess their antimicrobial properties. Biocatalytic and Electrochemical procedures were found to be more effective, safe, economical, environmental friendly, easy to handle. These green methodologies over conventional chemical methods provide new and improved synthetic routes to many valuable compounds.

Keywords: Aromatic alcohol, Biocatalysts (Baker's yeast), cyclic voltammetry, Constant current electrolysis, Glassy carbon electrode, Biocidal Studies.

INTRODUCTION

Stereo selective reduction of carbonyl moieties is a very useful tool for the introduction of stereogenic centres into chiral synthons, which are necessary for the preparation of natural products and pharmaceuticals[1]. Microbial biotransformation[2] processes for organic synthesis are advantageous because of the unique regio- and stereo- selective properties of enzymes and their capacity to operate in non-extreme conditions[3-4]. From an economic point of view yeasts have attracted wide attention as a potential catalyst in biotransformation processes because they are cheap and easy to obtain[5]. Living cells of *Saccharomyces cerevisiae* bioconvert carbonyl moiety into corresponding alcohol. Several parameters like volume ratio of aqueous over glycerol, pH of the medium and substrate concentration greatly influence the biocatalysis[6].

The electrochemical reduction is one of the greener approaches because it is pollution free as electrons are regarded as one of the reagents therefore it reduces the use of at least one hazardous chemical reagent. These reactions can take place in a low-temperature environment, reducing the local consumption of energy, the risk of corrosion, material failure, and accidental release[7-8]. Electrochemical techniques are also very useful to investigate kinetics and mechanisms of the reactions hence electro organic synthesis provide alternative synthetic route[9].

Vanillyl alcohol (4-hydroxy-3-methoxy-benzyl alcohol), which is prepared by the reduction of vanillin, is starting material for the synthesis of biologically active molecules, flavoring ingredients and is utilized by two insect species, namely African sugar-cane borer moth and the Leaffooted pine seed bug in its chemical communication system. Vanillyl alcohol has dopamine receptor stimulant which is used for treatment of Parkinson's disease[10]. Alkaline salt solution of Vanillyl alcohol i.e. potassium salt of Vanillyl alcohol which is hydroxyl-methoxy-sulfonic acid-benzyl alcohol (HMSBA) is used for treating cancerous tumors[11]. 1-(4-bromo-phenyl)-ethanol is a useful starting material in pharmaceutical industries for drug synthesis[12].

Apocynol (1-(4-hydroxy-3-methoxyphenyl) ethanol), a phenol derivative, is used as a simple model for lignin. Aromatic alcohols were proved to have biological activity against bacteria. Phenyl ethyl alcohol in relatively low concentrations exerts an effective inhibitory action on Gram-negative bacteria therefore use for differential inhibition[13].

In view of the above applications, the present work relates to the reduction of 4-bromo acetophenone, 4-hydroxy-3-methoxyacetophenone, 4-hydroxy-3-methoxybenzaldehyde to the corresponding aromatic alcohol such as 1-(4-Bromo phenyl) ethanol, 1-(4-Hydroxy-3-methoxyphenyl) ethanol and 4-Hydroxy-3-methoxybenzylalcohol employing Biocatalysis using Baker's Yeast (in a mixture of glycerol and water) and Electrochemical technique to evaluate electrode reaction using cyclic voltammetry and constant current electrolysis & reports the results of the undertaken antimicrobial evaluation.

MATERIAL AND METHODS

All chemicals used in the present investigation were of analytical grade. All the solvents were dried and then distilled out. Doubly distilled water was used to prepare the required solutions. ^{1}H NMR spectra were recorded using Joel (Japan) 300MHZ spectrophotometer. FT-IR spectra were recorded from Nicolet (USA) FT-IR spectrophotometer.

Biocatalytic Reduction

Immobilization of Baker's Yeast in 5% polyacrylamide gel has been carried out by preparing following solutions.

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

Solution B: - 5.98gm 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 gm Sucrose in 100ml doubled distilled water.

The above mention solutions were taken in following proportions:-

Solution A (10 ml)
Solution B (5 ml)
Solution C (5 ml)
Solution D (20 ml)

The solutions were then mixed in the following sequence:-

solution A+ solution B+ solution D+ Baker's Yeast (5 g) and solution C.

The resulting solution was then deareated & 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), 10 g fresh Baker's Yeast (free or immobilized), 10 g sucrose 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& Table-2). 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/melting point measurement and spectral techniques viz. IR, NMR (Table-1 & Table-2).

Table: 1 Physical & Spectral data from free Baker's Yeast mediated Reduction

Product Name	Reaction Time (in hrs)	Boiling/ Melting Point (^{0}C)	Free Baker's Yeast (%)	IR Data (cm^{-1})	NMR(δ-Value)
1-(4-bromophenyl)ethanol	72	120	71	3370(OH), 3008(Ar C-H str), 2960(CH-str), 1600,1495,1468(C=C ring str), 1200&1384(C-O str Ph), 1100&1280 (C-O str Sec.alcohol)	2.3(O**H**), 4.8(-C**H**-OH), 1.46(-C**H$_3$**), 7.2(ortho-C**H**), 7.4(meta-C**H**),
1-(4-Hydroxy-3-methoxy phenyl)ethanol	96	101	73	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**),
4-Hydroxy-3-methoxy benzyl alcohol	96	113	76	3365(OH), 3010(Ar C-H str), 2965(CH-str), 1600,1527,1465(C=C ring str), 1200&1380(C-O str Ph), 1050 (C-O str primary alcohol)	2.1(O**H**), 3.8(-OC**H$_3$**), 4.5(-CH-**OH**), 6.8(ortho-C**H**), 6.7(meta-C**H**),

Table: 2 Physical & Spectral data from Immobilized Baker's Yeast mediated Reduction

Product Name	Reaction Time (in hrs)	Boiling/ Melting Point (^{0}C)	Immobilized Baker's Yeast Yield (%)	IR Data (cm^{-1})	NMR(δ-Value)
1-(4-bromophenyl)ethanol	72	120	78	3355(OH), 3006(Ar C-H str), 2965(CH-str), 1603,1497,1465(C=C ring str), 1200&1390(C-O str Ph), 1100&1284(C-Ostr Sec.alcohol)	2.3(O**H**), 4.8(-C**H**-OH), 1.46(-C**H$_3$**), 7.2(ortho-C**H**), 7.4(meta-C**H**),
1-(4-Hydroxy-3-methoxy phenyl)ethanol	96	101	76	3360(OH), 3020(Ar C-H str), 2975(CH-str), 1607,1527,1470(C=C ring str), 1200&1374(C-O str Ph), 1100&1284(C-Ostr Sec.alcohol)	2.0(O**H**), 3.7(-OC**H$_3$**), 5.1(-CH-**OH**), 6.9(ortho-C**H**),
4-Hydroxy-3-methoxy benzyl alcohol	96	113	82	3355(OH), 3020(Ar C-H str), 2960(CH-str), 1600,1525,1468(C=C ring str), 1205&1386(C-O str Ph), 1055 (C-O str primary alcohol)	2.0(O**H**), 3.8(-OC**H$_3$**), 4.5(-CH-**OH**), 6.8(ortho -C**H**), 6.7(meta-C**H**)

Electrochemical Reduction

The completely computer controlled Basic Electrochemistry System model ECDA-001 was used for recording cyclic voltammograms of selected compounds 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 reactant was added to blank solution then initial potential, final potential, scan rate and current sensitivity were provided and the resulting current was measured as a function of applied potential.

Constant Current Electrolysis

Carbonyl Compounds were subjected to constant current electrolysis at constant current at 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 B.R. buffer of appropriate pH and the supporting electrolyte (CH3COONa) was filled in both the limbs of H cell. Carbonyl compound was dissolved in minimum amount of methanol and placed in the cathodic compartment and electrolysed at constant current (1.0 amp). After the completion of reaction, extraction was done with diethyl ether (30ml) and the procedure was repeated three times. The ether extract was dried over calcium chloride and then characterized by combined application of boiling/melting point measurement, chromatographic and spectral techniques (Table-3).

Table: 3 Physical & Spectral data from Electrochemical Reduction

Product Name	Reaction Time (in hrs)	Boiling/ Melting Point (^{0}C)	Electrochemical Yield (%)	IR Data (cm^{-1})	NMR (δ-Value)
1-(4-bromophenyl)ethanol	72	120	78	3365(OH), 3010(Ar C-H str), 2970(CH-str), 1605,1497,1470(C=C ring str), 1200&1386(C-O str Ph), 1100&1286(C-OstrSec.alcohol)	2.1(O**H**), 4.8(-C**H**-OH), 1.48(-C**H**$_3$), 7.2(ortho-C**H**), 7.4(metaC**H**),
1-(4-Hydroxy-3-methoxy phenyl)ethanol	96	101	76	3370(OH), 3015(Ar C-H str), 2980(CH-str), 1608,1527,1475(C=C ring str), 1200&1376(C-O str Ph), 1100&1290(C-OstrSec.alcohol)	2.0(O**H**), 3.8(-OC**H**$_3$), 4.9(-CH-**OH**), 6.9(-C**H**),
4-Hydroxy-3-methoxy benzyl alcohol	96	113	82	3360(OH), 3020(Ar C-H str), 2980(CH-str), 1602,1525,1468(C=C ring str), 1205&1380(C-O str Ph), 1050 (C-O str primary alcohol)	2.0(O**H**), 3.8(-OC**H**$_3$), 4.6(-CH-**OH**), 6.8(ortho-C**H**),

Antimicrobial activity

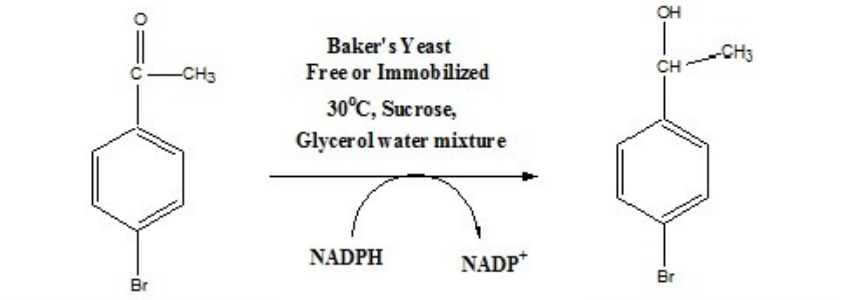
The *in vitro* biological screening effect of the compounds were tested against the bacteria *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeuruginosa* (ATCC 27853), *Enterococcus faecalis* (MTCC 439), by Well Diffusion Method. The microorganism were cultured in nutrient agar medium in Petri plates and used as inoculums for the study. Measured quantities of the test compounds were dissolved in Ethanol 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 (millimeter) of inhibitory zone around each disc was measured after 24 hours. Filter paper disc treated with Ethanol served as control and Streptomycin (2.5 mg) used as reference drugs.

RESULTS AND DISCUSSION

Biocatalytic reduction

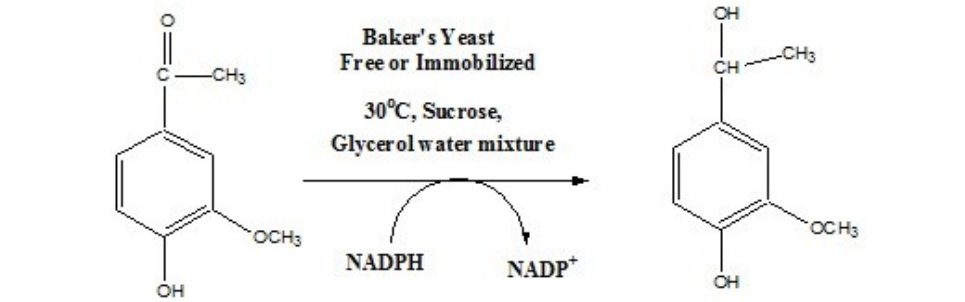
Biocatalytic reduction of 4-Bromoacetophenone, 4-Hydroxy-3-methoxyacetophenone, and 4-Hydroxy-3-methoxybenzaldehyde has been carried out as shown by the schemes below.

Scheme 1



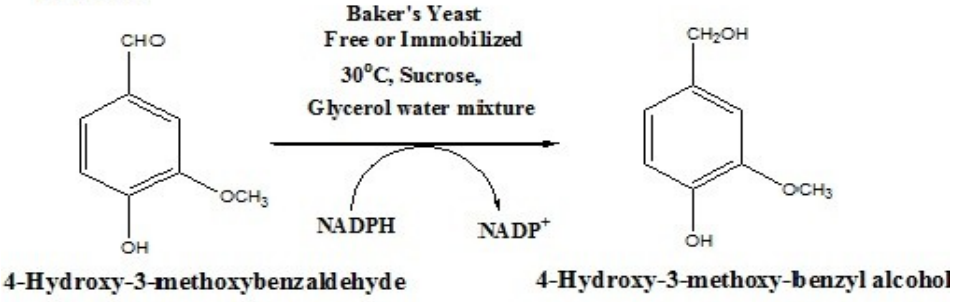
1-(4-Bromophenyl)-ethanone — 1-(4-Bromophenyl)-ethanol

Scheme 2



1-(4-Hydroxy-3-methoxy-phenyl)-ethanone — 1-(4-Hydroxy-3-methoxyphenyl)-ethanol

Scheme 3



4-Hydroxy-3-methoxybenzaldehyde — 4-Hydroxy-3-methoxy-benzyl alcohol

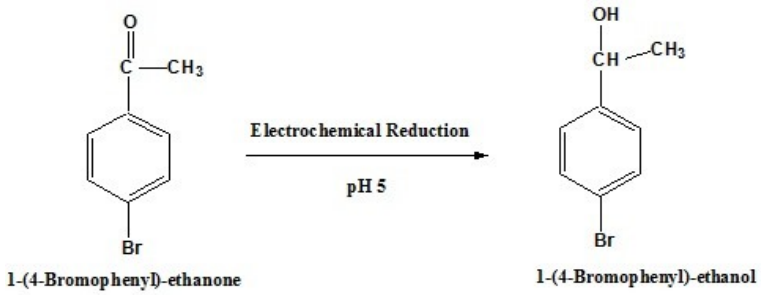
Asymmetric reduction of carbonyl compounds using whole cells of Baker's Yeast as biocatalysts involve two enzyme systems. One of them is the enzyme catalyzing the asymmetric reduction and other is the cofactor regeneration system, which supplies NADPH from $NADP^+$ through the oxidation of the energy source such as carbohydrates. *Saccharomyces cerevisiae* cells has an extra cellular invertase (β-D-fructosidase), that 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 and natural solvent for biocatalysis from the viability and activity point of view, glycerol is an alternative green solvent. 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 FBY, isolation of the products and repeated reused.

Electrochemical Reduction

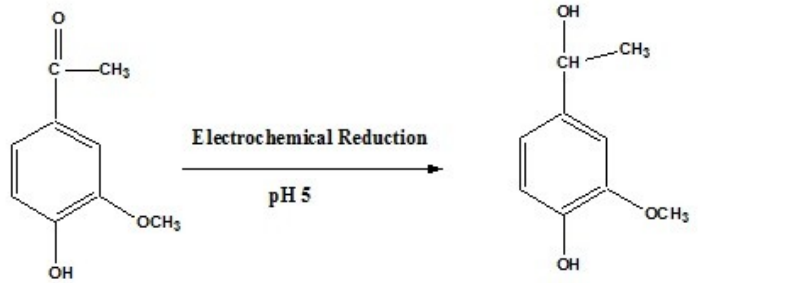
Electrochemical reduction of 4-Bromoacetophenone, 4-Hydroxy-3-methoxyacetophenone, and 4-Hydroxy-3-methoxybenzaldehyde has been carried out as shown by the schemes below.

Scheme 4



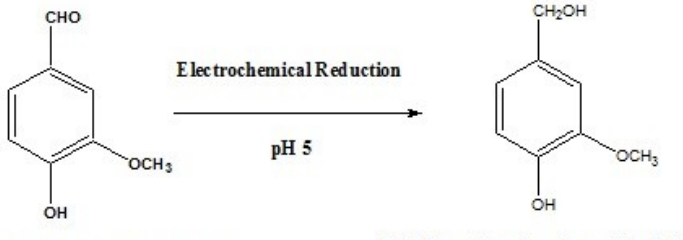
1-(4-Bromophenyl)-ethanone — 1-(4-Bromophenyl)-ethanol

Scheme 5



1-(4-Hydroxy-3-methoxy-phenyl)-ethanone — 1-(4-Hydroxy-3-methoxyphenyl)-ethanol

Scheme 6



4-Hydroxy-3-methoxy-benzaldehyde — 4-Hydroxy-3-methoxybenzylalcohol

In the cyclic voltammograms of 4-Bromoacetophenone, 4-Hydroxy-3-methoxacetophenone and 4-Hydroxy-3-methoxybenzaldehyde single irreversible cathodic peak was observed due to the reduction of >C=O moiety to the corresponding secondary alcohol. Kinetic Parameters evaluated from cyclic voltammograms are given in Table 4.

Table: 4 Voltammetric data evaluated from cyclic voltammograms at pH 5.0

Compound	v (mV/s)	E_{pc}(mV)	I_{pc}(µA)	Ip/√v
4-Bromoacetophenone	100	-590	228	22.80
	200	-632	320	22.85
	300	-664	389	22.88
	400	-667	468	23.4
	500	-688	517	23.59
4-Hydroxy-3-methoxyacetophenone	100	-482	148	14.8
	200	-505	209	14.92
	300	-514	256	15.05
	400	-534	309	15.45
	500	-541	350	15.90
4-Hydroxy-3-methoxybenzaldehyde	100	-515	137	13.70
	200	-526	204	14.46
	300	-546	254	14.68
	400	-553	295	14.75
	500	-562	344	15.40

Effect of pH

The influence of pH on reduction process was examined. On increasing pH the reduction peak shifts towards more negative values as shown in Fig.1, 2 and 3. The observed shift in E½ with decreasing pH to more positive values can be explained by protonation of the carbonyl group, thus easing the reduction. This dependence indicates that there is a proton transfer in the electrode reactions.

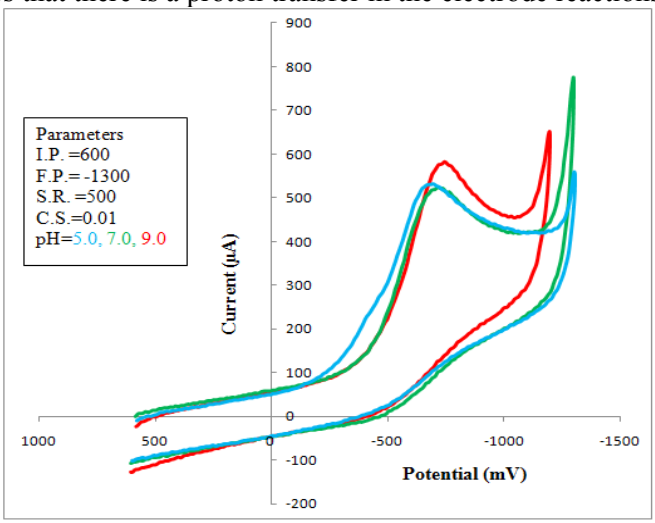


Fig.1 Cyclic voltammogram of 4-Bromoacetophenone at different pH

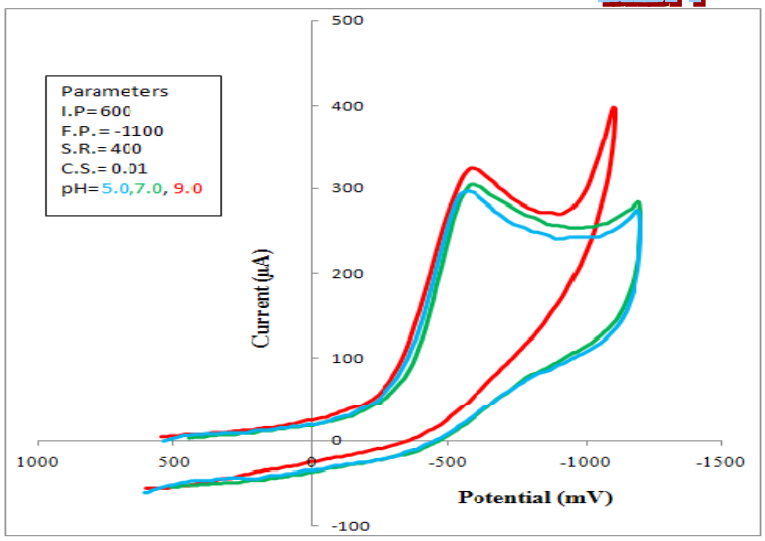


Fig.2 Cyclic voltammogram of 4-Hydroxy-3-methoxyacetophenone at different pH

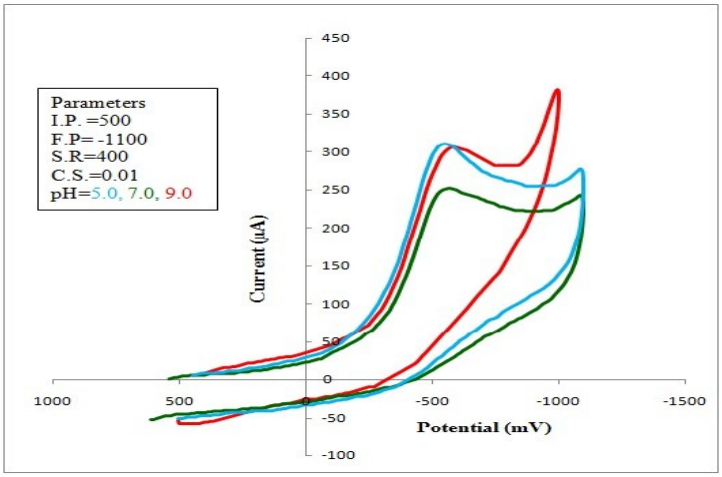


Fig.3 Cyclic voltammogram of 4-Hydroxy-3-methoxybenzaldehyde at different pH

Effect of scan rate

The effect of scan rate on peak current potential (Epc) was also studied. Fig.4, 5, and 6, shows the effect of scan rate on Epc at pH 5.0. On increasing the scan rate the peak current potential (Epc) is shifted towards more negative potentials indicating an irreversible electron transfer process. The dependence of the voltammetric peak current (Ipc) of the wave on the square root of scan rate ($v^{1/2}$) is linear with correlation coefficients (0.997,0.996,0.996) close to unity (graph 1) at all the pH[14] . Under these conditions the current process was diffusion controlled. Thus 4-Bromoacetophenone, 4-Hydroxy-3-methoxyacetophenone and 4-Hydroxy-3-methoxybenzaldehyde were reduced electrochemically in a diffusion -controlled irreversible cyclic voltammetry wave.

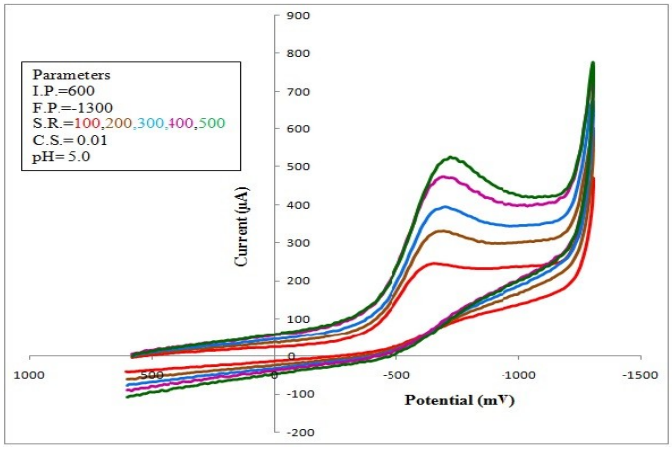


Fig.4 Cyclic voltammogram of4-Bromoacetophenone at various scan rates

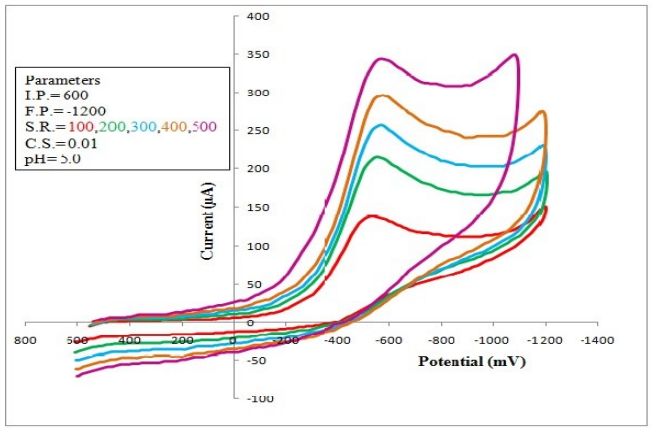


Fig.5 Cyclic voltammogram of4-Hydroxy-3-methoxyacetophenone at various scan rates

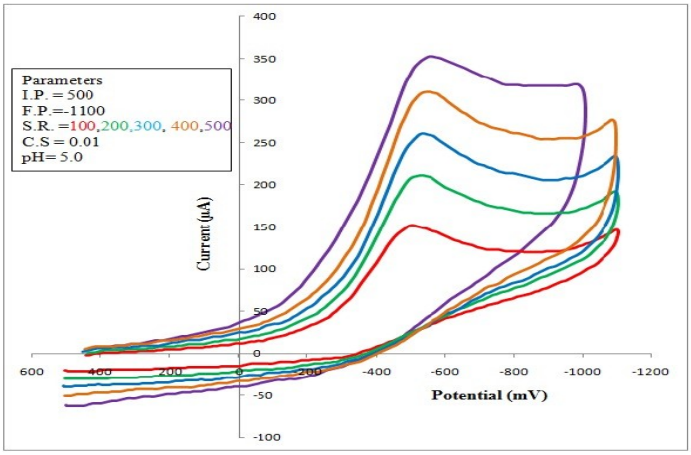
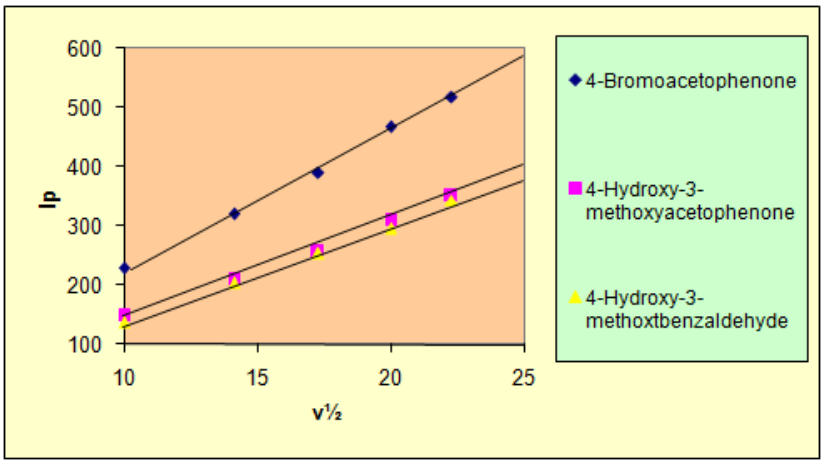


Fig.6 Cyclic voltammogram of4-Hydroxy-3-methoxybenzaldehyde at various scan rates



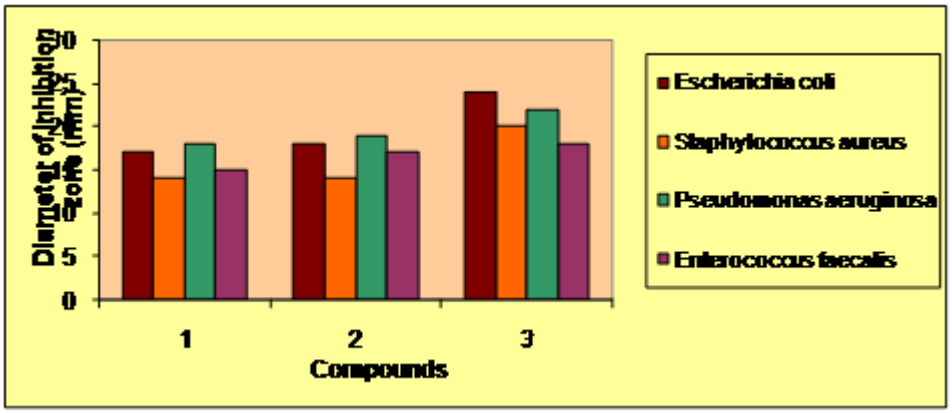
Graph 1.Variation of the cathodic peak currents (Ip) with $v^{1/2}$ for of 4-Bromoacetophenone, 4-Hydroxy-3-methoxyacetophenone and 4-Hydroxy-3-methoxybenzaldehyde at pH 5.0

Antimicrobial activity

The all synthesized aromatic alcohols such as 1-(4-bromo phenyl) ethanol, 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. From the results (Table 5), it is concluded that all of the tested compounds exhibit moderate antimicrobial activity against all species of bacteria used in this study.

Table: 5 Antimicrobial screening data of the Aromatic alcohols

S.No.	Aromatic alcohols	Diameter of inhibition zone (mm)			
		Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*
1.	1-(4-bromo phenyl)ethanol	17mm	14mm	18mm	15mm
2.	1-(4-Hydroxy-3-methoxy phenyl)ethanol	18mm	14mm	19mm	17mm
3.	4-Hydroxy-3-methoxy benzyl alcohol	24mm	20mm	22mm	18mm



Graph 2. Statistical representation for biological activity of1 (1-(4-Bromo phenyl) ethanol), 2(1-(4-Hydroxy-3-methoxyphenyl) ethanol) and 3(4-Hydroxy-3-methoxybenzylalcohol)

Conclusion

Aromatic alcohols have been prepared and characterized on the basis of analytical and spectral data. These alcohols were prepared using Biocatalytic and Electrochemical procedures which follows green methodology over conventional chemical methods in terms of effectiveness, safety, economy, ecofriendly nature, easy to handle and provide new and improved synthetic routes to many valuable compounds in fields of pharmaceutical, flavor and perfume industry. Furthermore, all the compounds are found to be potential bioactive material against the pathogenic microorganism.

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