Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article **DOI - 10.26479/2018.0401.07**

SYNTHESIS, ANTIMICROBIAL AND ELECTROCHEMICAL STUDY WITH SnO_2 NANO PARTICLE MODIFIED CARBON PASTE ELECTRODE OF ETHY L 6 METHYL-2-OXO-4-PHENYL-1, 2, 3,4-TETRAHYDROPYRIMIDINE-5 CARBOXYLATE DERIVATIVES

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ABSTRACT: 1, 2, 3, 4-Tetrahydropyrimidine (DHPM) is well known moiety with various biological activities. Synthesis of 1, 2, 3, 4-tetrahydropyrimidine carboxylate was achieved by Biginelli's reaction and characterized by spectroscopic techniques. The entitled molecules were studied on Tin dioxide /stannic oxide (SnO_2) nanoparticle modified carbon paste electrode (MCPE) in presence of phosphate buffer solution as supporting electrolyte by cyclic voltammetry techniques. The CV studies of all the analytes showed that the SnO_2 modified CPE has better electro catalytic activity towards the analytes in comparison to Bare CPE. The synthesized compounds were screened for their antimicrobial activity against Gram-negative bacterias (*Escherichia coli* and *Staphylococcus aureus*) and fungi such as *Candida albicans* and *Penicillin chrysogenum* and found to possess considerable antimicrobial activity suggesting their potency in development of novel antibiotics.

KEYWORDS: Cyclic voltammetry, Carbon paste electrode, Nitrogen heterocycles, antimicrobial activity

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1.INTRODUCTION

Nitrogen hetrocycles are very important class of heterocycles in drug-discovery studies. These compounds perform a crucial role in cell physiology and are efficient intermediates for several biological processes [1, 2]. The utilization of electrochemical cells to generate oxidation and reduction profiles, drug stability experiments, quantitative analyses, and *in vitro* experiments of drug candidates has been thoroughly investigated in the recent years [3, 4]. In the last decade, the synthesis

of Biginelli compounds such as esters of 1, 2, 3, 4-tetrahydropyrimidine-5-carboxylic acids has been of significant research attention [5-7]. The one-step, three-component Biginelli condensation [8-10] and the multi-stage Atwal procedures [11-14] are popular synthetic procedures that provide access to the pyrimidines. It is envisaged that the substituent effect may be attributable to intramolecular hydrogen association between alkoxy oxygen and the proton of pyrimidine ring NH group [15]. A number of marine alkaloids having dihydropyrimidine core unit have exhibited significant biological activities like antiviral, antimicrobial and anti-inflammatory performances [16]. Several functionalized derivatives are employed as calcium channel blockers and antihypertensive agents [17]. Suitable clinical diagnosis of recurrent microbial diseases continues to be a challenging task to clinicians and researchers across the globe mainly due to increase in multidrug resistance and antibiotic associated toxicities [18]. These alarming issues have led to the unremitting exploration for novel compounds with antimicrobial activities [19]. Though past two decades have witnessed significant progress in terms of exploration of synthetic and semi synthetic antibacterial compounds, synthesis of potent antimicrobial agent is focus of the present day research [20]. Electrochemical methods are well established techniques for various analytical studies especially for the analysis of drugs and pharmaceuticals owing to their high sensitivity, versatile, low detection limits as well as inexpensive instrumentations. These modalities involve the direct conversion of chemical information into signals in terms of current, potential and charge [21]. Electrochemical processes are highly efficient in assaying the concentration of electro active analyte at trace levels and beneficial in obtaining information regarding its physical and chemical characters like oxidation potential, diffusion coefficients, electron transfer rates and electron transfer number can be obtained. Besides, these methods are of utmost interest in the investigation of pharmacologically active compounds and metabolites produced/synthesized by different metabolic pathways involving redox reactions [22]. Voltammetry are a set of analytical techniques employed for the evaluation of properties of analytes by studying the activity of analyte to an electrical charge in a potential range. This technique is a function of continuous change of the potential applied across the electrode-solution interface and resultant current is noted. The resulting voltammamogram is comparable to a conventional spectrum in a way that it gives out information as a variable of energy scan. Voltammetric techniques like potential step, linear sweep, differential pulse, square wave, stripping and cyclic voltammetry are used for the determination of redox potential of the synthesized molecules [5, 6, 23]. Thus, in the pursuit for compounds with redox potential, CV studies of synthesized molecules were found worth exploring for their pharmaceutical applications. However, there are no studies on the electrochemical (CV) and biological activities of these derivatives so far. To the best of our knowledge, researches on cyclic voltametry and antimicrobial activities of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate derivatives have not been reported. In the present study, derivatives of 1, 2, 3, 4-Tetrahydropyrimidine (DHPM) was synthesized and characterized. The cyclic

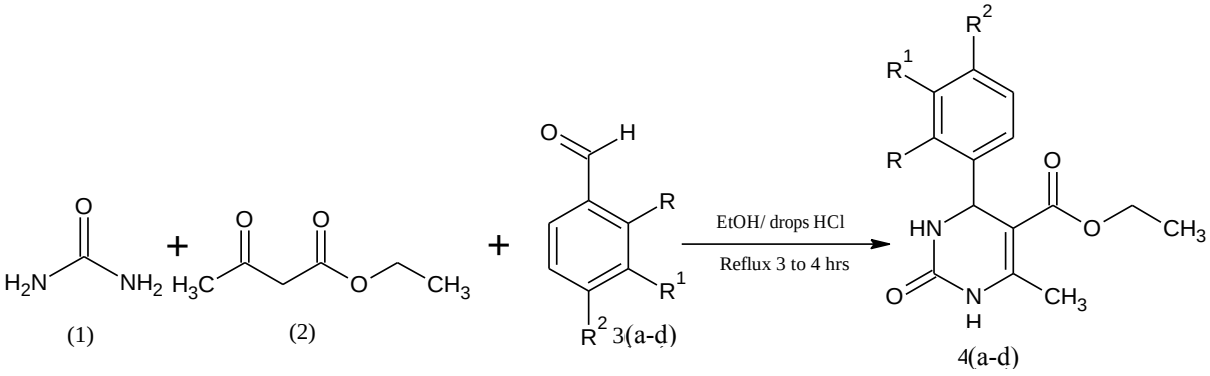
voltammetric studies on SnO_2nanoparticle modified carbon paste electrode (MCPE) were carried out. These derivatives were also checked for their antimicrobial potential. Against this background, in the present study detailed information on structure of the title compounds have been established and the outcomes are discussed here.

2. MATERIALS AND METHODS

2.1 Synthesis

General procedure for the synthesis of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4(a-d)

According to the reported method [19] a mixture of ethyl acetoacetate (0.130 g, 1 mmol), benzaldehyde (0.106 g, 1 mmol) and urea (0.070 g, 1.17 mmol) was refluxed in ethyl alcohol for about 3-4 hrs. The reaction mixture was cooled for overnight. The precipitate was subjected to filtration followed by washing with water and ethanol to obtain white solid (0.29 g, 91 % yield; mp 204-206).



R	R^1	R^2
-H-	-H-	-H-
-H-	CF_3	-H-
-H-	-H-	-OH
-H-	OH	-H-

Figure 1: General scheme for the preparation of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-

tetrahydropyrimidine-5-carboxylate 4(a-d)

Chemicals of analytical grade were utilized in the current study. 2-naphthol and dimethylformamide were procured from Spectrochem. The purity of the as-prepared compounds was assessed by TLC. The melting point was found using open capillary tubes based on Prefit model. In the present investigation, NMR spectra was recorded on JEOL AL 300, 300.4 MHz FT NMR spectrometer with $CDCl_3$/DMSO as solvent and presented relative to TMS internal standard.

2.2 Electrochemical Studies

Reagents and stock solutions

Tin (II) chloride dehydrate ($SnCl_2.H_2O$, 99.99 %, Merck), Nitric acid (HNO_3, 70 %, Merck) Citric acid ($C_6H_8O_7$, 99.5 %, Merck) and L-tyrosine, potassium chloride (KCl) were procured from Merck and rest of the chemicals were of AR grade except spectroscopically pure graphite powder.

The electro polymerisation was carried out in 0.2 M phosphate buffer. The phosphate buffer solution (PBS) was prepared from dihydrogen sodium phosphate and di potassium hydrogen phosphate (KH_2PO_4 and K_2HPO_4) and the pH was maintained using 0.1 N NaOH solution. 10 mM stock solution of ethyl 6-methyl-2-oxo-4-phenyl- 1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4(a-d) (analyte) was prepared by dissolving it in water. 1 M potassium chloride (KCl) was utilized as supporting electrolyte for all analytes. All solutions were prepared with double distilled water.

Apparatus

Electrochemical determinations were performed on a model-201 electrochemical analyzer (EA-201 chemlink systems) in a conventional three-electrode assembly. Carbon paste electrode, having a cavity of 3 mm diameter is employed as working electrode, platinum electrode and saturated calomel electrode (SCE) were utilized as auxiliary electrode and standard reference electrode respectively.

Preparation of SnO_2 nanoparticles

SnO_2 nanoparticles were prepared by gel combustion method. The raw material used was tin (II) chloride dehydrate. 6.2 mole of nitric acid was used as oxidizing agent and added in suitable ratio to form a tin nitrate solution, then 1.5 mole of citric acid which acts as fuel was mixed with solution, and solution was heated to 90 °C in a Pyrex vessel with continuous stirring. When temperature was increased to about 300 °C, the polymeric precursor underwent a strong, self-sustaining combustion reaction with evolution of huge volume of gases and swelled into voluminous and foamy ashes. The entire combustion process takes place in few seconds. The produced ashes were then calcined at 800 °C (for 1 h). The process was continued until the complete breakdown of carbonaceous residues. Then the white powder of SnO_2 nanoparticles were collected [24].

Preparation of bare carbon paste electrode

The bare carbon paste electrode was prepared by hand mixing of graphite powder 70 % and silicon oil 30 % in an agate mortar for about 30 min in order to obtain homogenous carbon paste. The paste was then packed into the cavity of a Teflon tube electrode (3 mm diameter). Before analysis, modified

electrode was smoothened on a piece of transparent paper to get a uniform, smooth and fresh surface.

Preparation of SnO_2 nanoparticles modified carbon paste electrode

SnO_2 nanoparticles modified carbon paste electrode was prepared by hand mixing of 70 % graphite powder and 10 mg SnO_2 nanoparticle with 30 % silicon oil in an agate mortar get a homogenous carbon paste. The paste was packed into the cavity (3 mm in diameter) and then smoothened on a weighing paper. Copper wire connected to the paste in the end of the tube served as electrical contact. This modified electrode was immersed in PBS (pH 6.5) and electrochemical measurements were carried out in a voltammetric cell in the potential range from 500 mV to 1200 mV by using cyclic voltammetric technique. The same method was followed for all the sample analysis at room temperature [24].

2.3 **Antimicrobial activity**

The *in vitro* antibacterial and antifungal activities of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4(a-d) derivatives were tested against gram negative bacterias *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (25923), and fungi *Penicillium crysogenium* and *Candida albicans* by agar disc diffusion method. The microbial strains were cultured overnight at 37 ˚C in nutrient broth and potato dextrose agar medium respectively. The broth cultures were compared to the turbidity with that of the standard 0.5 McFarland solution. All the Micro-organisms were stored at 4˚C for future use. Ciprofloxacin and Amphotericin were utilized as standard drugs for bacteria and fungi respectively. The agar plates of the media (Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water-bacteria and Czapek-Dox Agar: Composition (g/l) Sucrose-30.0; Sodium nitrate- 2.0; K_2HPO_4-1.0, $MgSO_4$. $7H_2O$-0.5; KCl-0.5; $FeSO_4$-0.01; Agar-20-fungi) were prepared and wells were made in the plate. Each plate was inoculated with minimum of 18 hrs old cultures (100 µl, 10^{-4} CFU) and spread evenly on the plate. After 20 min, the wells were filled with the compounds and antibiotic of different concentrations. All the plates were subjected to incubation at 37 ºC for 24 hrs for bacteria and at 27 ºC for 96 hrs for fungi.The zone of inhibition around the well in each plate was measured. The lowest concentration of the compounds fully hindering the growth of bacteria and fungi compared against standard antibiotic was recorded as minimum inhibitory concentration **[**25-27**]**.

3.RESULTS AND DISCUSSION

3.1Synthesis of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-Carboxylate4(a-d)

Figure 1 shows the scheme for synthesis of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5- carboxylate 4(a-d) derivatives from ethyl acetoacetate, benzaldehyde and urea with 91% yield and melting point of 204-206.

3.2 *In vitro* antimicrobial study

The synthesized compounds were screened for their antimicrobial activity. The results are tabulated in Table-1, 2, 3 and 4. The MIC values of the synthesized compounds are shown. The antibacterial results revealed that compound 4d was the most effective against *Escherichia coli and Staphylococcus aureus*, with MIC values ranging from 250 to 1000 µg/ml. Compounds 4(a-d) showed less significant activity against fungi *Candida albicans* and *Penicillin chrysogenum* with MIC values of more than 1000 µg/ml, respectively. *C. albicans* were resistant to synthesized drug. Figure 2 and 3 are representative pictures of zone of inhibition against *E. coli* and *S. aureus* as given by synthesized analogues of ethyl 6- methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4(a-d).

Table-1: Zone of inhibition against *E. coli* as given by synthesized analogues of ethyl 6- methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4(a-d)

Samples	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
4a	0	0	0	0	0	0	NF
4b	0	0	0	0	0	6	1000
4c	0	0	0	5	8	12	250
4d	0	0	0	0	0	6	1000
Ciprofloxacin	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
	26	29	32	34	38	*	25

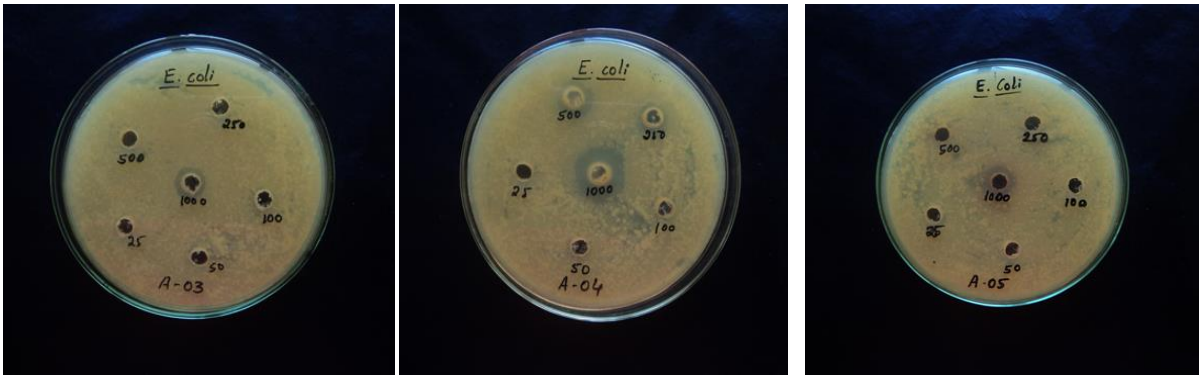


Figure 2: Representative picture of zone of inhibition as given by the analogues of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4 (b, c, d) against *E. coli*

Table-2: Zone of inhibition against *S. aureus* as given by synthesized analogues of ethyl 6- methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4(a-d)

Samples	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
4a	0	0	0	0	0	0	NF
4b	0	0	0	0	0	0	1000
4c	0	0	0	6	9	14	250

4d	0	0	0	0	0	5	1000
Ciprofloxacin	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
	30	32	34	35	38	*	25

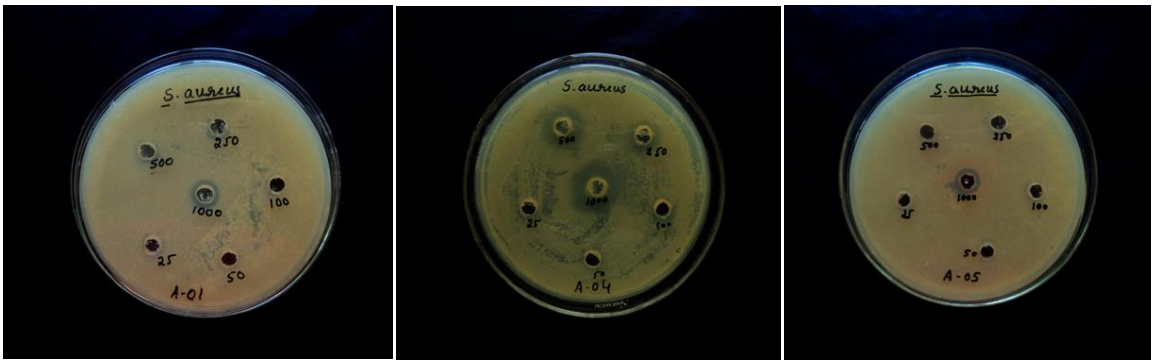


Figure 3 : Representative picture of zone of inhibition as given by the analogues of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4(a, c, d) against *S. aureus*

Table-3: Zone of inhibition against *C. albicans* as given by synthesized analogues of ethyl 6- methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4 (a-d)

Samples	**25 µg**	**50 µg**	**100 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
4a	0	0	0	0	0	0	NF
4b	0	0	0	0	0	0	NF
4c	0	0	0	0	0	0	NF
4d	0	0	0	0	0	0	NF
Amphotericin	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
	0	0	5	9	13	15	100

Table-4: Zone of inhibition against *P. chrysogenum* as given by synthesized analogues of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate 4(a-d)

Samples	**25 µg**	**50 µg**	**100 µg**	**250 µg**	**500 µg**	**1000 µg**	**MIC µg**
4a	0	0	0	0	0	0	NF
4b	0	0	0	0	0	0	NF
4c	0	0	0	0	0	0	NF
4d	0	0	0	0	0	0	NF
Amphotericin	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
	0	0	0	0	3	5	500

Note: In above tables, NF is MIC not found in the concentrations screened *zones could not be measured due to merging, Zones ≥3 mm considered for MIC

The MIC of the synthesized compounds were compared to standard antibacterial drug ciprofloxacin and antifungal drug Amphotericin. Our study demonstrated the significant antibacterial effect and mild antifungal activity suggesting wide range of antimicrobial potential against gram positive, gram

negative bacterias and fungi.

3.3 Cyclic Voltammetric Studies

Electrochemical responses of ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate 4(a-d) derivatives at BCPE and MCPE with SnO_2 Nano particle

Cyclic voltammetry is often the first experiment carried out in an electrochemical investigation based on potential control and most widely used for obtaining qualitative data about electrochemical processes. It is largely applicable in the study of redox reactions, detection of reaction intermediate and products formed at the electrode and for the study of pharmaceuticals and biologically active compounds. The cyclic voltammograms obtained for the electrochemical responses were recorded in a 0.2M phosphate buffer as the supporting electrolyte at pH 7.2. SnO_2 Nano particle modified carbon paste electrode (SNP-MCPE) was used as working electrode in presence of phosphate buffer solution. The results of cyclic voltammetric studies are shown in Figure- 4, 5, 6 and 7. The results showed well-defined redox peaks at SNP-MCPE. Results indicated that the oxidation peak current of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate derivatives was relatively enhanced at SnO_2 nano particles modified CPE than bare carbon paste electrode.

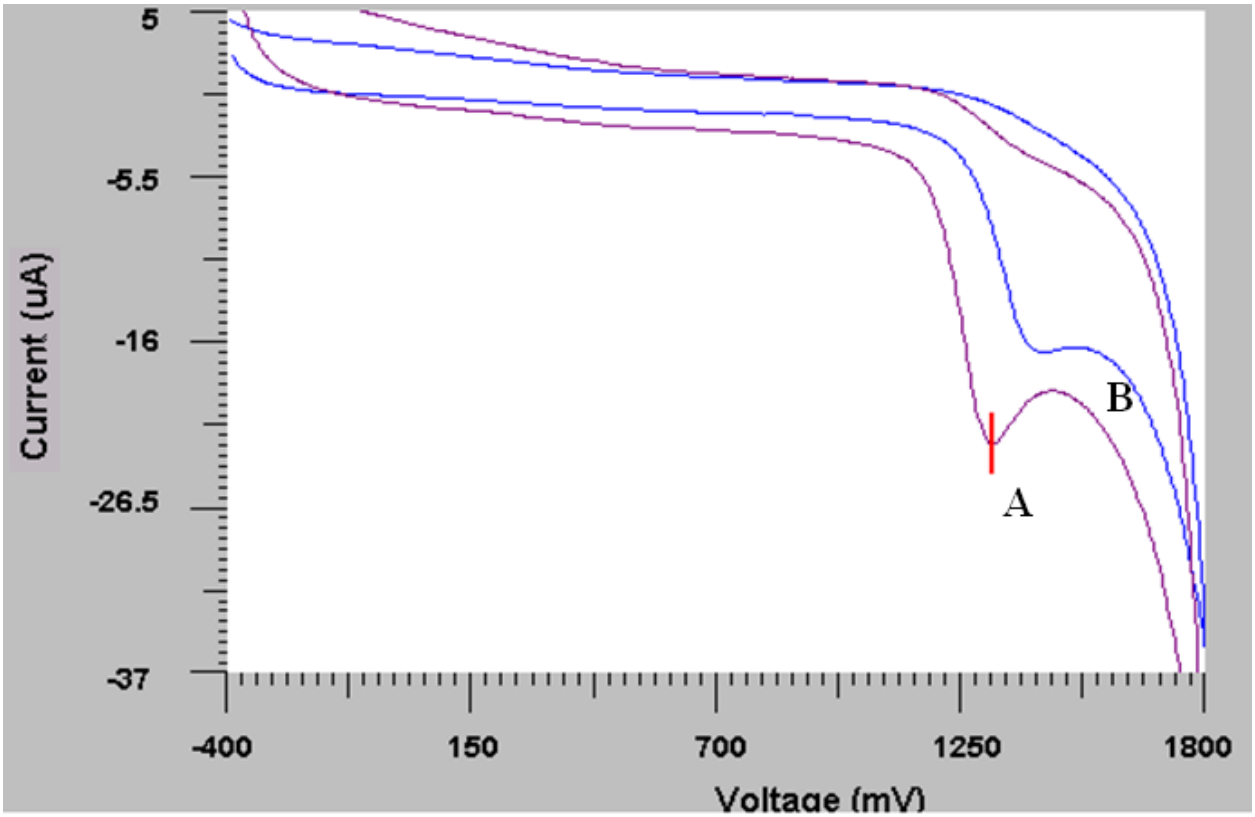


Figure-4: Cyclic Voltammogram of analyte 4a at SnO_2 Nano particle MCPE in 0.2 M Phosphate buffer solution. (**Curve A**- SnO_2 Nano particle MCPE, **Curve B** -Bare Carbon Paste electrode, Initial

Potential 400 mV, Terminal potential: 1800mV, Scan rate: 50 mVs^{-1})

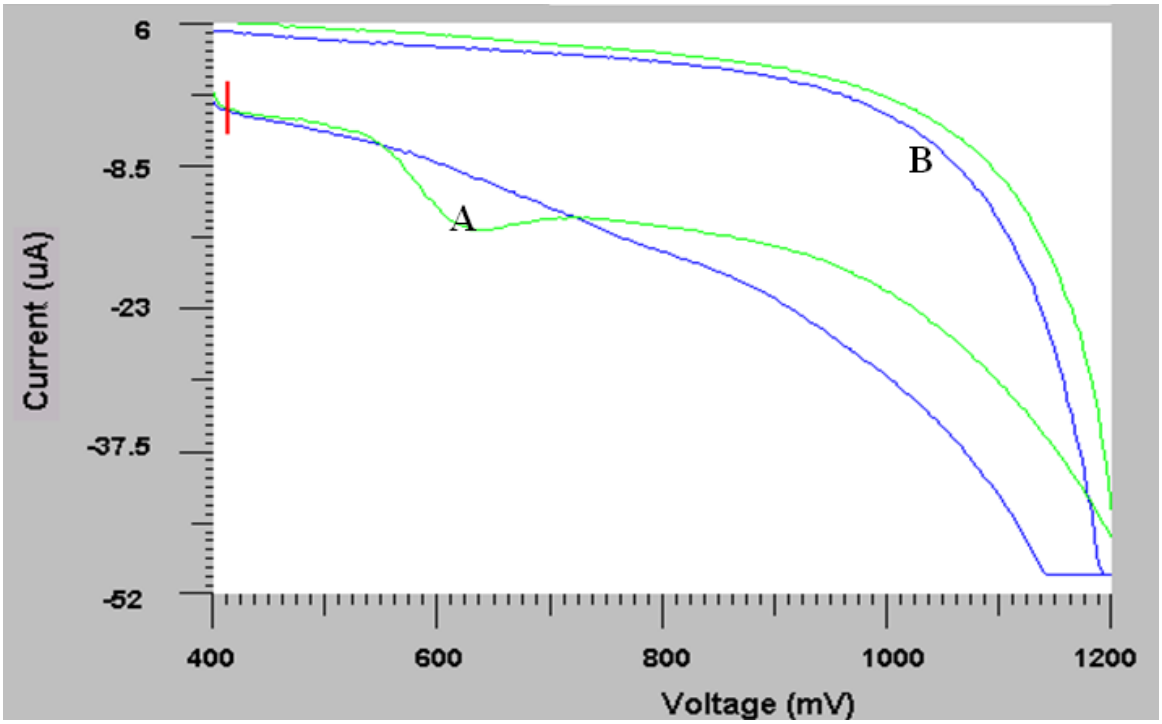


Figure-5: Cyclic Voltammogram of analyte 4b at SnO_2 Nano particle MCPE in 0.2 M Phosphate buffer solution. (**Curve A** - SnO_2 Nano particle MCPE, **Curve B** -Bare Carbon Paste electrode, Initial Potential 400 mV, Terminal potential: 1200mV, Scan rate: 50 mVs^{-1})

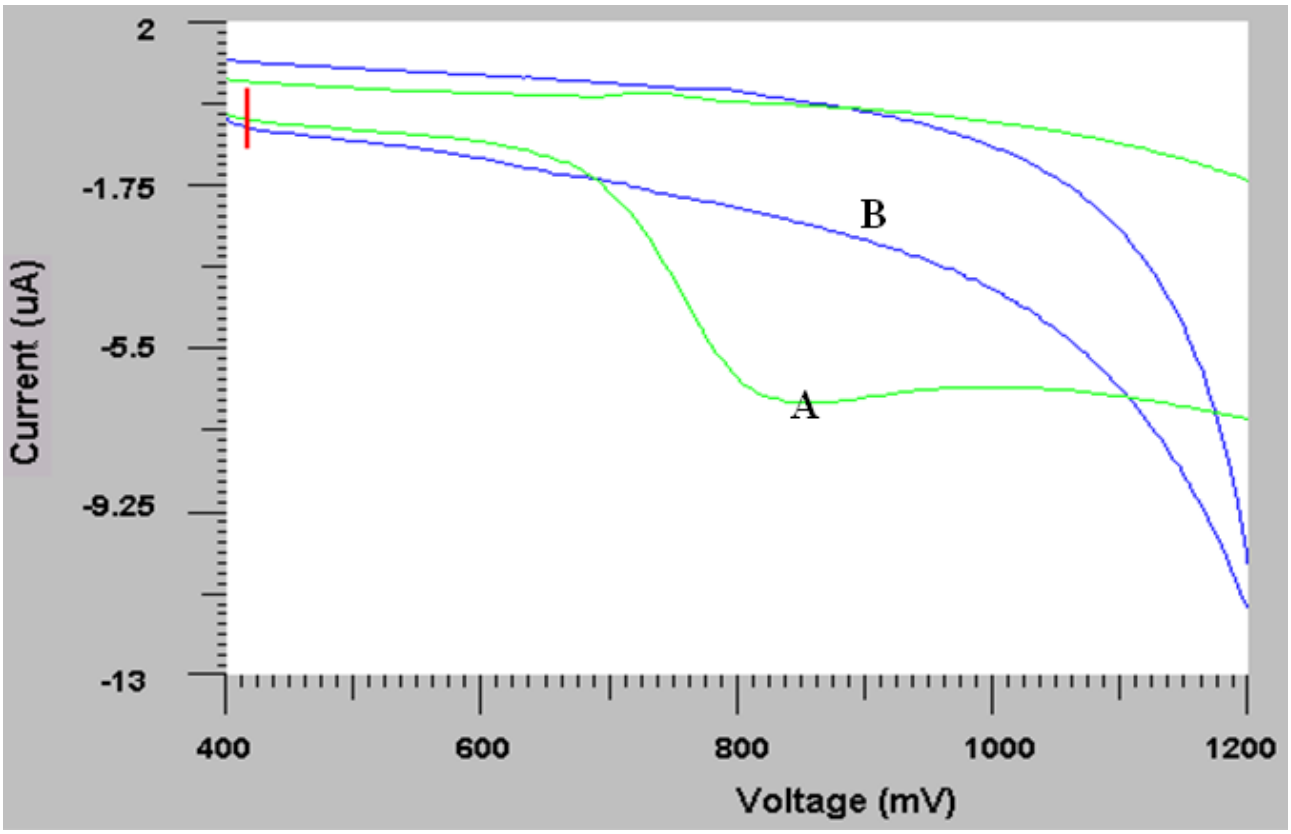


Figure-6: Cyclic Voltammogram of 4c analyte at SnO_2 Nano particle MCPE in 0.2 M Phosphate

buffer solution at a scan rate of 50 mV s^{-1}. (**Curve A** - SnO_2 Nano particle MCPE, **Curve-B** -Bare Carbon Paste electrode, Initial Potential 400 mV, Terminal potential: 1200 mV, Scan rate: 50 mVs^{-1})

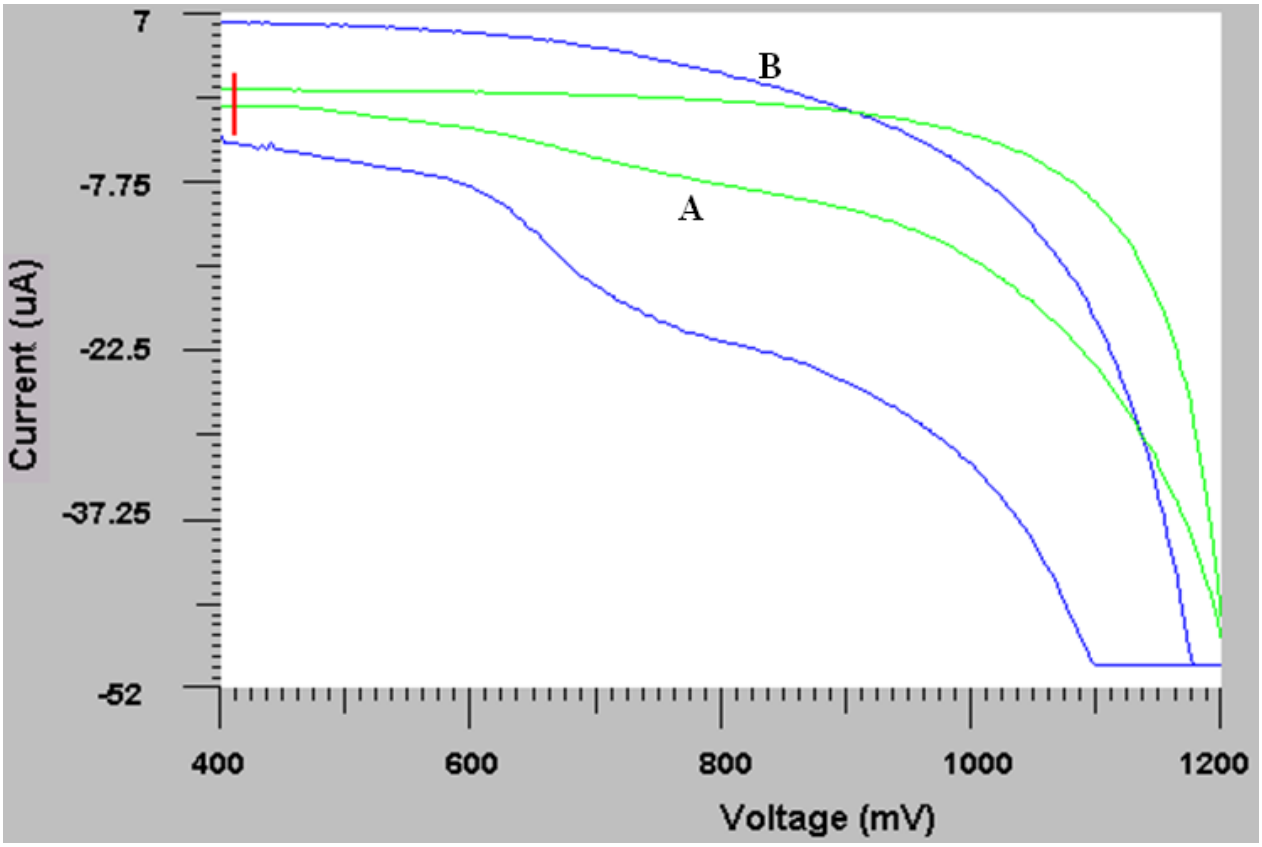


Figure-7: Cyclic Voltammogram of analyte 4d at SnO_2 Nano particle MCPE in 0.2 M Phosphate buffer solution. (**Curve A** - SnO_2 Nano particle MCPE, **Curve B** -Bare Carbon Paste electrode (BPCE), Initial Potential 400 mV, Terminal potential: 1200 mV, Scan rate: 50 mVs^{-1})

Figure 4 show the cyclic voltammograms obtained for analyte 4a at a scan rate of 50 mV/s in the potential window 400 to 1800 mV. The cyclic Voltammogram showed irreversible peak at potential 1300 mV and 1400 mV of peak current 20 µA and 60 µA for modified and BCPE respectively. This indicates that SnO_2 Nano particle MCPE exhibited high sensitivity/ detection compared to BPCE. Similarly, CV studies for analyte 4b at a scan rate of 50mV/s in the potential window of 400 to 1200 mV. Figure 5 shows the cyclic Voltammogram obtained for both bare CPE and modified CPE. It is found that, only in case of modified CPE, irreversible peak for the analyte was noticed. In all the cases the enhancement of peak current has been observed in modified electrode compared to bare carbon paste electrode. Also for rest of the analytes, that is from 4c to 4d, cyclic voltammetry studies were carried out and the obtained graphs are shown in figure 6 and 7. It is noticed that in these cases also a irreversible peak for analytes are obtained and the peak current for analyte is higher in case of SNP-MCPE. From these it can be deduced that the SnO_2 modified CPE has better electrocatalytic activity towards the analytes in comparison to bare CPE.

4. CONCLUSION

In the present study, we described one pot synthesis of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-

tetrahydropyrimidine-5-carboxylate derivatives via the reaction of substituted aldehydes, ethylacetoacetate and ammonium acetate using ethanol as a solvent. The antimicrobial studies revealed that the compounds from 4a to 4d showed antimicrobial activity with 4d exhibiting highest activity. Further, the analysis was extended to electrochemical studies by SnO_2 nano particles modified carbon paste electrode for the electrochemical determination of ethyl 6-methyl-2-oxo-4-phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate derivatives. Results showed that the oxidation peak current of the synthesized compound and its derivatives was enhanced at SnO_2 nano particles modified CPE. The electrochemical response is diffusion controlled and irreversible in nature.

ACKNOWLEDGEMENT:

The authors are thankful to Kuvempu University for providing facilities to carry out the present work.

CONFLICT OF INTEREST:

The authors have no conflict of interest to declare.

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