Research Article

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Cyclic Voltammetric Behaviour of The Ethanol Extracts of *Pisonia Grandis(R.Br)*

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Abstract: Antioxidants help organisms deal with oxidative stress, caused by free radical damage. In the present study, ethanol extract of *Pisonia grandis* and its 90% ethanol fractionate were subjected to electrochemical analysis by cyclic voltammetry using KCl as supporting electrolyte at glassy carbon electrode at various concentrations and scan rates. Both the extracts had a reversible and an irreversible redox reaction. The electrochemical response is directly related to the structure of the antioxidant and the potential required for its oxidation.

Keywords : cyclic voltammetry, *Pisonia* , glassy carbon, antioxidants

INTRODUCTION:

The ethanolic extract of *Pisonia grandis* (synonym *Pisonia alba*) has antidiabetic activity, anti-inflammatory activity, wound healing, and anti-ulcer properties **[1].** This might be due to the secondary metabolites present in the plant. Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Electrochemical approaches are of special advantage in studies of the antioxidant properties. The cyclic voltammetry procedure reported by **Kohen *et al*** [2] evaluates the overall reducing power of low molecular weight antioxidants in a biological fluid or tissue homogenate. DPPH assay of *Pisonia grandis* has revealed significant free radical scavenging power, representative of its potent antioxidant nature [3-4]. The aqueous fraction of *Pisonia grandis* shows good antioxidant potential as seen from cyclic voltammetric studies at glassy carbon electrode [5]. The free radical scavenging activity of *Pisonia grandis* has been attributed probably to the hydrogen-donating ability of its constituents [6]. In the present study, the ethanol extract of *Pisonia grandis* and its 90% ethanol fractionate were subjected to electrochemical analysis by cyclic voltammetry using KCl as supporting electrolyte at glassy carbon electrode.

MATERIALS AND METHODS:

The leaves of *Pisonia grandis* were collected from Coimbatore, South India. The leaves were washed, air dried, pulverized and refluxed with ethanol for six hours. The ethanol extract obtained was filtered, concentrated and fractionated. The ethanol extract (ET) and 90% ethanol fraction (NE) were analysed using cyclic voltammetry to study its oxidation potential and hence its antioxidant activity.

Instrumentation: The experimental set up for CV measurement consisted of a Solartron model number 1280 ZT electrochemical system (1284 B + USB 128087S) – CIF analyzer controlled by a personal computer with the Corrware program. Calculations were done using Corrview software.

Electrochemical Cell: Cyclic voltammetric experiments were performed using a three electrode system consisting of a 3 mm diameter glassy carbon (MF 2012) as working electrode , saturated calomel as reference electrode and a platinum counter electrode immersed in a small glass cell with provision for inserting electrodes and nitrogen purging. All potentials are referred to the reference electrode. All the electrodes are polished and rinsed before the start of the experiment.

Preparation of sample: About 1g of each crude extracts (ET and NE) was weighed and refluxed with 25 ml of extraction medium (water/ acetonitrile/ acetic acid 20: 15: 15) for 3 hours and the filtrate was refrigerated for further use.

Recording the cyclic voltammograms of the extracts: The extract(2ml) was pipetted out into a small glass container and neutralized to pH ~7 using phosphate buffer. To this 5 ml of 0.5M KCl solution was added as the supporting electrolyte and cyclic voltammograms were recorded. The tracings were recorded from a potential range of -2.5V to +2.5V at scan rates of 120, 100, 50, 20 and 10 mV/s and at various concentrations with pH 6-7.

Variation of scan rate: Influence of scan rate on peak potential and peak current was studied. For each concentration of the extracts the cyclic voltammogram was recorded at various scan rates (10mV/s, 20mV/s, 50mV/s, 100mV/s, and 120mV/s) with KCl as supporting electrolyte.

Variation of concentration : Effect of concentration on the peak current and potential was studied by varying concentration of extracts. The concentration was increased by adding an additional 1ml (~40 mg/ml) of the prepared sample and the solution stirred. Phosphate buffer was added to adjust pH whenever necessary. Five different concentrations were prepared. Samples of various concentrations of ET and NT were designated as ET1, ET2, ET3, ET4, ET5 and NT1, NT2, NT3, NT4, NT5 respectively.

RESULTS AND DISCUSSION:

An analysis of the cyclic voltammograms of the extracts recorded at various concentrations and scan rates reveal that during the forward scan, reduction (cathodic peak) occurred and during the reverse scan, oxidation (anodic peak) occurred. The parameters noted were anodic peak potential (Ea), anodic peak current (Ia), cathodic peak potential (Ec) and cathodic peak current(Ic). The electrochemical response is directly related to the structure of the antioxidant and the potential required for its oxidation. This can be an excellent option for all antioxidants that usually present redox properties [7].

Preliminary phytochemical screening of the ethanol extract revealed the presence of tannins, saponins, steroids and phenolic constituents. These might be the components undergoing redox

reactions. Recently the insulinomimetic pinitol and kerolytic allantoin have been reported from the ethanol extract of this plant in our laboratory [8]. These constituents along with the other reported metabolites might be contributing to the antioxidant activity of the extracts.

Effect of scan rate: The ethanolic extract showed two anodic (0.3 to 0.6V-Ea1) (1.6 to 1.9V-Ea2) and one cathodic peak (0.4 to 0.7V-Ec) at all scan rates and at all concentrations (**Table 1-5**). A representative cyclic voltammogram is shown in figure 1. Peaks at 0.3-0.6V (anodic) (Ea1) appeared symmetrical to cathodic peak at 0.4-0.7V, which may be attributed to a reversible redox reaction. But an extra anodic peak at 1.6-1.9V may indicate an irreversible oxidation process. These peaks may indicate the presence of some components in ethanol extract that undergo a reversible oxidation process and some other that undergo an irreversible oxidation. The anodic peak potential (Ea), cathodic peak potential (Ec), anodic peak current (Ia) and cathodic peak current (Ic) increases with increase in scan rate (**Figure 2,3**). The linear increase in Ia with √SR indicates that the oxidation process is diffusion controlled [9].

All the cyclic voltammograms of NE have two anodic and one cathodic peak at all scan rates and at all concentrations. The anodic peaks are 0.4 to 1 V (Ea1) and 1.8-2V (Ea2). The cathodic peak appears at 0.5- 0.8V (Ec) (**Table 6-10**). A representative CV is shown in **figure 4.** The anodic peak at Ea1 and the cathodic peak Ec are symmetrical and may indicate a reversible redox reaction. With increase in scan rate Ea, Ec, Ia and Ic increased (**Figures 5,6).** The anodic peak Ea2 may indicate that the generated product may be rapidly removed during the process. The anodic current increases linearly with increase in concentration and scan rate. This may be attributed to diffusion process.

Effect of concentration: Cyclic voltammetric behaviour of ethanol extract at various concentrations is shown in **Figure 7**. With increase in concentration of the extract an increase in anodic peak potential (Ea) was noted. The cathodic peak potential (Ec) increases till the third concentration and thereafter there is deviation from linearity (**Table 1-5**).The anodic peak current (Ia) decreases with increase in concentration. The linear increase in Ia with √SR indicates that the oxidation process is diffusion controlled **[9].** A high anodic current (112.84 x 10^{-5} Amp/cm^2) is obtained for ET2 at scan rate 120 mV/s. With increasing concentration and scan rate there is decrease in anodic and cathodic peak current and peak potential. This kind of shift with increasing concentration indicates that the product molecules are adsorbed over the electrode surface **[10].**

Cyclic voltammograms of NE recorded at various concentrations and scanned at 100mV/s are shown in figure 8. The anodic peak potential shows many deviations with increase in concentration (**Table 6-10**). A high anodic current is obtained for NE1 at scan rate 100 mV/s (424.69 10^{-5} Amp/cm^2).

CONCLUSION:

The ethanol extract and 90% ethanol fractionate of *Pisonia grandis* were analysed electrochemically by cyclic voltammety at glassy carbon electrode. Both the extracts express a reversible and an irreversible redox reaction. The antioxidant potentials of the extract are indicators of its antioxidant activity. Cyclic voltammetry can be effectively used as a tool to analyze the antioxidant potentials of plant extracts.

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Table 1: Cyclic peak parameters obtained for the ethanol extract ET1 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.3780	Ia1	5.6869	0.6632	5.256	1.754
	Ea2	1.68	Ia2	36.924			
20	Ea1	0.5491	Ia1	8.989	0.7145	9.0766	1.301
	Ea2	1.7295	Ia2	56.072			
50	Ea1	0.6403	Ia1	14.099	0.7350	11.348	1.148
	Ea2	1.7698	Ia2	72.023			
100	Ea1	0.7158	Ia1	17.847	0.7712	18.015	1.077
	Ea2	1.8196	Ia2	89.473			
120	Ea1	0.4955	Ia1	18.735	0.4619	16.845	0.932
	Ea2	1.8291	Ia2	95.347			

Table 2: Cyclic peak parameters obtained for the ethanol extract ET2 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.447	Ia1	3.4042	0.4144	1.5767	0.9263
	Ea2	1.709	Ia2	20.948			
20	Ea1	0.508	Ia1	5.6586	0.5029	3.1239	0.9894
	Ea2	1.739	Ia2	32.289			
50	Ea1	0.589	Ia1	10.883	0.5344	9.0305	0.9062
	Ea2	1.782	Ia2	59.131			
100	Ea1	0.606	Ia1	20.098	0.7011	22.752	1.1568
	Ea2	1.816	Ia2	92.832			
120	Ea1	0.606	Ia1	25.021	0.5616	19.487	0.9265
	Ea2	1.841	Ia2	112.84			

Table 3: Cyclic peak parameters obtained for the ethanol extract ET3 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.4771	Ia1	2.2961	0.416	1.245	0.8734
	Ea2	1.7287	Ia2	15.607			
20	Ea1	0.4975	Ia1	3.5366	0.442	2.0969	0.8900
	Ea2	1.7489	Ia2	26.375			
50	Ea1	0.5578	Ia1	5.1332	0.493	4.2376	0.8840
	Ea2	1.8076	Ia2	53.001			
100	Ea1	0.6082	Ia1	8.0123	0.563	7.2987	0.9260
	Ea2	1.8331	Ia2	79.451			
120	Ea1	0.6681	Ia1	9.0773	0.623	9.9963	0.9336
	Ea2	1.8829	Ia2	84.82			

Table 4: Cyclic peak parameters obtained for the ethanol extract ET4 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.5472	Ia1	1.7824	0.4659	1.1481	0.8515
	Ea2	1.7321	Ia2	13.135			
20	Ea1	0.5674	Ia1	2.7947	0.5326	2.043	0.9388
	Ea2	1.7491	Ia2	23.67			
50	Ea1	0.5878	Ia1	4.65	0.6226	4.2741	1.0594
	Ea2	1.7921	Ia2	43.989			
100	Ea1	0.6181	Ia1	7.2002	0.6829	7.7643	1.1049
	Ea2	1.8234	Ia2	69.928			
120	Ea1	0.6480	Ia1	8.1209	0.7429	8.6435	1.1465
	Ea2	1.8495	Ia2	68.813			

Table 5: Cyclic peak parameters obtained for the ethanol extract ET5 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.5066	Ia1	1.8687	0.4759	1.1932	0.9393
	Ea2	1.742	Ia2	12.156			
20	Ea1	0.5575	Ia1	2.8363	0.5427	2.1966	0.9735
	Ea2	1.7694	Ia2	26.266			
50	Ea1	0.5775	Ia1	5.4799	0.5729	3.931	0.9920
	Ea2	1.8576	Ia2	48.683			
100	Ea1	0.6083	Ia1	6.4955	0.6037	5.7395	0.9924
	Ea2	1.8622	Ia2	53.479			
120	Ea1	0.6786	Ia1	9.0085	0.6542	10.859	0.9640
	Ea2	1.9568	Ia2	86.487			

Table 6: Cyclic peak parameters obtained for the 90% ethanol extract NE1 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.4869	Ia1	2.2057	0.7527	2.9908	1.5459
	Ea2	1.9494	Ia2	375.33			
20	Ea1	0.5874	Ia1	3.5363	0.7925	3.8297	1.3492
	Ea2	1.9592	Ia2	377.13			
50	Ea1	0.7683	Ia1	6.1514	0.8131	6.4733	1.0583
	Ea2	1.999	Ia2	388.48			
100	Ea1	0.9686	Ia1	8.7831	0.8433	9.7766	0.8706
	Ea2	2.0587	Ia2	424.69			
120	Ea1	1.1089	Ia1	9.0394	0.8933	11.045	0.8056
	Ea2	2.0983	Ia2	405.71			

Table 7: Cyclic peak parameters obtained for the 90% ethanol extract NE2 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.5870	Ia1	2.4021	0.6328	2.5558	1.0950
	Ea2	1.9022	Ia2	125.65			
20	Ea1	0.6572	Ia1	3.8981	0.7226	3.5409	1.0842
	Ea2	1.9685	Ia2	150.69			
50	Ea1	0.6991	Ia1	9.3631	0.7538	9.2444	1.0782
	Ea2	2.0083	Ia2	203.08			
100	Ea1	0.7699	Ia1	13.868	0.7412	19.837	1.0016
	Ea2	2.0216	Ia2	128.74			
120	Ea1	0.7661	Ia1	19.159	0.5315	20.214	0.6938
	Ea2	2.1279	Ia2	285.18			

Table 8: Cyclic peak parameters obtained for the 90% ethanol extract NE3 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.4571	Ia1	1.8533	0.6926	2.3555	1.5152
	Ea2	1.8301	Ia2	65.048			
20	Ea1	0.5570	Ia1	2.483	0.6628	2.9726	1.1899
	Ea2	1.8607	Ia2	84.768			
50	Ea1	0.7574	Ia1	3.9492	0.8424	4.7799	1.1122
	Ea2	1.931	Ia2	111.48			
100	Ea1	0.7594	Ia1	13.118	0.8603	15.661	1.1329
	Ea2	1.988	Ia2	157.56			
120	Ea1	0.7694	Ia1	12.905	0.7313	16.261	0.9505
	Ea2	2.0276	Ia2	156.6			

Table 9: Cyclic peak parameters obtained for the 90% ethanol extract NE4 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.2497	Ia1	0.9664	0.2048	0.66959	0.8202
	Ea2	1.7556	Ia2	90.147			
20	Ea1	0.3374	Ia1	1.6515	0.2062	1.2762	0.6112
	Ea2	1.8321	Ia2	95.621			
50	Ea1	0.7679	Ia1	5.0828	0.3536	3.8325	0.4605
	Ea2	1.9007	Ia2	102.3			
100	Ea1	0.8381	Ia1	7.7045	0.4037	6.9356	0.4817
	Ea2	1.9705	Ia2	103.98			
120	Ea1	0.8589	Ia1	10.162	0.5240	9.4611	0.6101
	Ea2	2.0374	Ia2	173.92			

Table 10: Cyclic peak parameters obtained for the 90% ethanol extract NE5 of *Pisonia grandis* at different scan rates

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm^2		Ec V	Ic 10^{-5} Amp/cm^2	Ec/Ea
10	Ea1	0.5212	Ia1	1.1807	0.2647	1.1854	0.5079
	Ea2	1.9003	Ia2	55.143			
20	Ea1	0.6524	Ia1	2.516	0.3182	2.1945	0.4877
	Ea2	1.9297	Ia2	61.876			
50	Ea1	0.7079	Ia1	3.816	0.3930	3.1241	0.5552
	Ea2	1.9453	Ia2	76.365			
100	Ea1	0.7983	Ia1	7.4261	0.4238	6.3465	0.5309
	Ea2	1.9507	Ia2	107.44			
120	Ea1	0.9089	Ia1	10.315	0.4939	8.8357	0.5434
	Ea2	1.9611	Ia2	129.29			

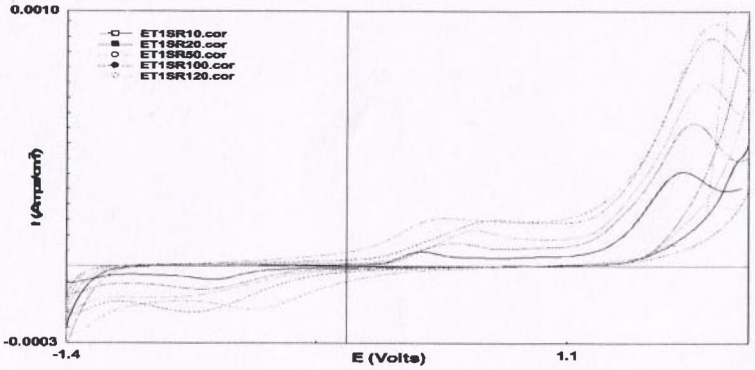
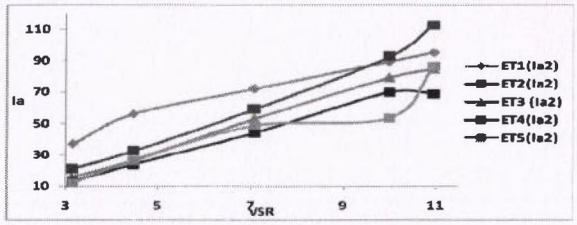
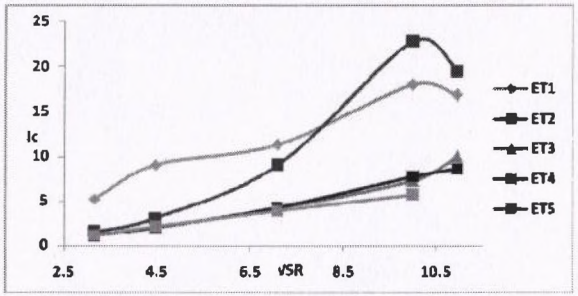


Figure 1. Cyclic voltammogram obtained for the ethanol extract ET1 of *Pisonia grandis* at different scan rates



y = 7.027x + 19.85	y = 11.49x - 18.35	y = 9.124x - 13.22
R^2 = 0.974	R^2 = 0.989	R^2 = 0.997
y = 7.571x - 10.09	y = 7.945x - 11.25	
R^2 = 0.986	R^2 = 0.891	

Figure 2. Effect on anodic (Ia) current of ethanol extract of *Pisonia grandis* at various scan rates and concentrations



y = 1.540x + 1.121	y = 2.728x - 8.265	y = 0.986x - 2.260
R^2 = 0.950	R^2 = 0.932	R^2 = 0.992
y = 1.058x - 2.577	y = 0.661x - 0.818	
R^2 = 0.959	R^2 = 0.998	

Figure 3. Effect on cathodic (Ic) current of ethanol extract of *Pisonia grandis* at various scan rates and concentrations

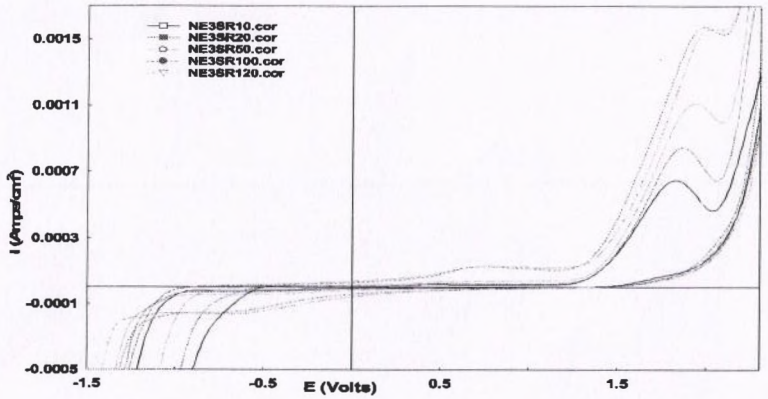
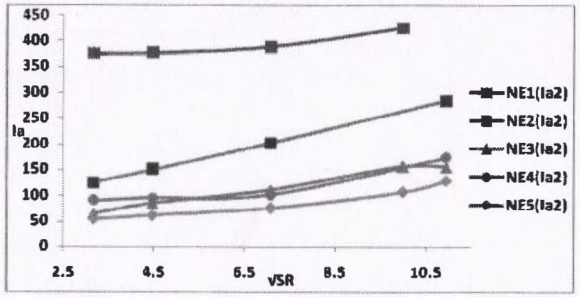


Figure 4. Cyclic voltammogram obtained for the 90% ethanol extract NE3 of *Pisonia grandis* at different scan rates at room temperature



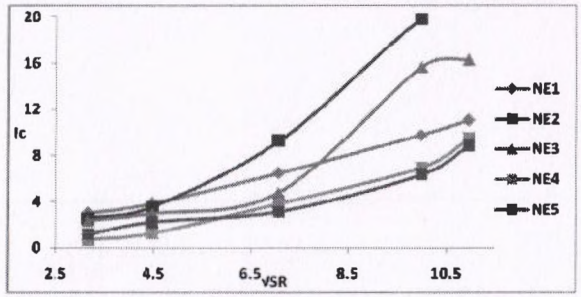
$y = 7.203x + 346.9$ $y = 12.26x + 27.64$ $y = 9.068x + 21.34$

$R^2 = 0.900$ $R^2 = 0.986$ $R^2 = 0.948$

$y = 20.52x + 59.49$ $y = 10.67x + 47.05$

$R^2 = 0.999$ $R^2 = 0.871$

Figure 5. Effect on anodic (Ia) current of 90% ethanol extract of *Pisonia grandis* at various scan rates and concentrations



$y = 1.046x - 0.641$ $y = 2.496x - 6.727$ $y = 1.960x - 5.573$

$R^2 = 0.994$ $R^2 = 0.971$ $R^2 = 0.906$

$y = 1.089x - 3.336$ $y = 0.903x - 2.102$

$R^2 = 0.967$ $R^2 = 0.924$

Figure 6. Effect on cathodic (Ic) current of 90% ethanols extract of *Pisonia grandis* at various scan rates and concentrations

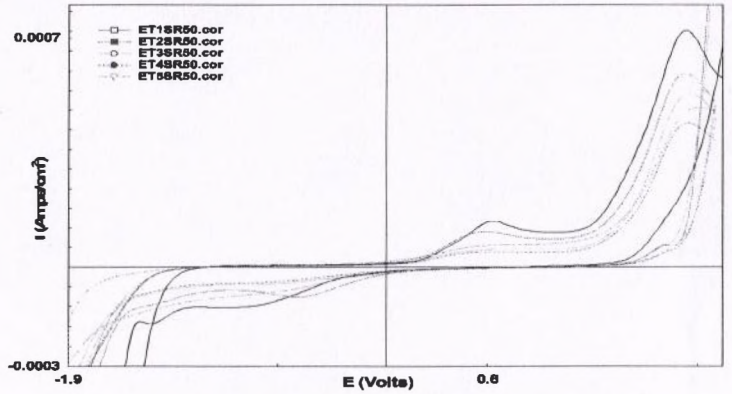


Figure 7. Cyclic voltammogram obtained for the ethanol extracts of *Pisonia grandis* at different concentration at scan rate 50mv/sec

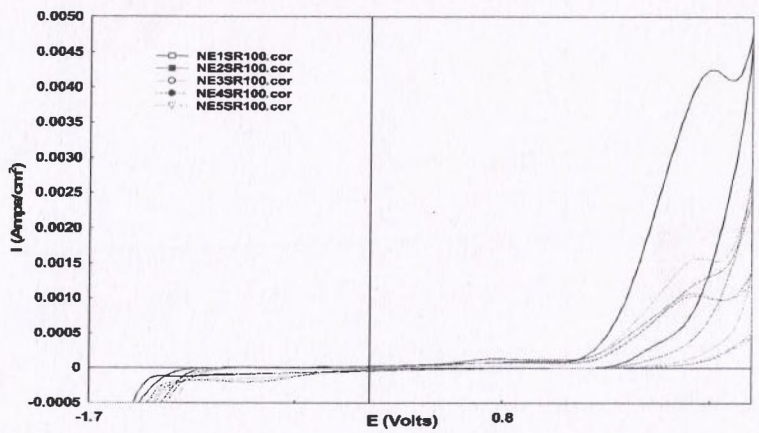


Figure 8. Cyclic voltammogram obtained for the 90% ethanol extracts of *Pisonia grandis* at different concentrations at room temperature

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