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RESEARCH ARTICLE

Electrochemical Behavior of the Aqueous Fraction of the Ethanol Extract of *Pisonia grandis* (R.Br) at Glassy Carbon Electrode

Shubashini K. Sripathi, Subashree and Lalitha P.*
Department of Chemistry, Avinashilingam Deemed University for Women, Coimbatore-641043.Tamilnadu
*Corresponding Author E-mail: goldenlalitha@gmail.com

ABSTRACT:
Pisonia grandis (R.Br) (grand devil's claw) (*Nyctaginaceae*) is a lettuce tree having anti-inflammatory, antidiabetic, diuretic, and wound healing activity. Antioxidant activity of aqueous fraction of ethanol extract of *Pisonia grandis* (R.Br) was analyzed by its electrochemical behavior. Cyclic voltammetry of the aqueous fraction of the ethanol extract at glassy carbon electrode with KCl as supporting electrolyte revealed a reversible and an irreversible redox reaction which is due to the presence of one or more secondary metabolites like flavonoids, phenols and tri-terpenoids having antioxidant properties

KEYWORDS: *Pisonia grandis*, glassy carbon electrode, antioxidant activity, cyclic voltammetry.

INTRODUCTION:
Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability[1]. Plants contain high concentrations of numerous redox-active antioxidants, such as polyphenols, carotenoids, tocopherols, glutathione, ascorbic acid and enzymes with antioxidant activity, which fight against hazardous oxidative damage of plant cell components[2]. Measurement of reducing capacity and electrochemical behavior of compounds may provide useful information about the free radical scavenging activity of naturallly occurring compounds. Methanol extract of *Pisonia grandis* has ability to scavenge free radicals by ability to inhibit lipid peroxidation[3]. The present study is aimed at testing the antioxidant capacity of ethanol extract of *Pisonia grandis* (R.Br) through cyclic voltammetric methods at glassy carbon electrode.

Experimental:
Antioxidant activity of aqueous fraction of ethanol extract of leaves of *Pisonia grandis*(R.Br) was analysed by cyclic voltammetry (CV). Leaves of *Pisonia grandis* was collected from Coimbatore district.

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The leaves were washed, air dried under controlled conditions and then pulverized. The dried pulverized leaves of *Pisonia grandis* were thoroughly percolated and refluxed with ethanol for about six hours. The ethanol extract obtained was filtered, concentrated and fractionated with 1:1 $CHCl_3$ water mixture. The aqueous portion (AQ) was collected separately and analysed for 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 the 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 and cyclic voltammetry analysis of the extract:
2 ml of the solution was pipetted out into a small glass container and neutralized to a pH of 7.0 using phosphate buffer. 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 a scan rate of 120, 100, 50, 20 and 10 mV/s at different concentrations and pH range 6-7.

Variation of scan rate:

Influence of scan rate on peak potential and peak current was studied. Aqueous fraction of *Pisonia grandis* (AQ) was analyzed by varying concentration. For each concentration of the extract the cyclic voltammogram was recorded at various scan rates (10mV/s, 20mV/s, 50mV/s, 100mV/s, and 120mV/s)

Variation of concentration:

Effect of concentration on the peak current and potential was studied by varying concentration of the aqueous extract of *Pisonia grandis*. The concentration was increased by adding 1 ml (~40 mg/ml) of the sample prepared and stirred by magnetic stirrer. Phosphate buffer was added to adjust pH whenever necessary.

RESULTS AND DISCUSSION:

Cyclic voltammetric analysis of aqueous fraction of ethanol extract of *Pisonia grandis:*

All the cyclic voltammograms recorded for the fractionated aqueous portion of ethanol extract of *Pisonia grandis* (R.Br) showed 2 anodic (0.3-0.9V (Ea1)) (1.7-1.9V (Ea2)) and 1 cathodic peak (0.5- 0.9V(Ec)).

Effect of scan rate:

Influence of scan rate on peak potentials and currents was studied by varying the scan rate (10mV/s, 20mV/s, 50mV/s, 100mV/s, and 120mV/s) with KCl as supporting electrolyte at glassy carbon electrode.

Cyclic voltammograms of aqueous extracts of *Pisonia grandis* at all concentrations (AQ1,AQ2, AQ3, AQ4, AQ5) and at all scan rates showed 2 anodic peaks at 0.3-0.9 V (Ea1) and 1.7-1.9 V (Ea2) and 1 cathodic peak at 0.5-0.9 V. **(Table 1-5).** A representative cyclic voltammogram of variation of scan rate at concentration AQ5 is shown in the **Figure 1.**

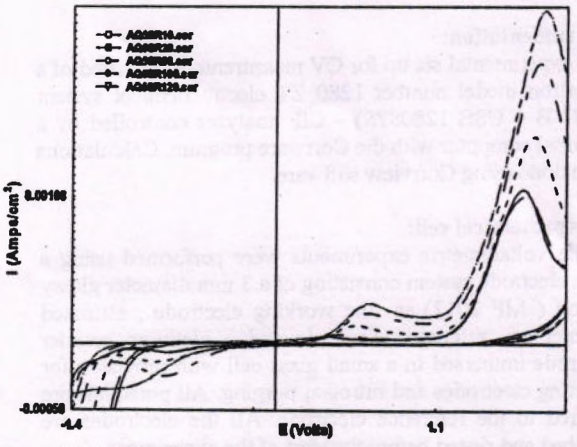


Figure 1: Cyclic voltammogram obtained for the aqueous extract AQ5 of *Pisonia grandis* at different scan rates at room temperature

Peaks at 0.3-0.9V (anodic) (Ea1) appeared symmetrical to cathodic peak at 0.4-0.7V, which may be attributed to a reversible redox reaction. The Ia/Ic value for all concentration at all scan rates was ≈1. This adds to the proof for reversible process. But an extra anodic peak at 1.7-1.8 may indicate an oxidation process that was followed by a chemical reaction which rapidly removed the generated product [2]. These peaks may indicate presence of few some components in aqueous extract (AQ) that undergo reversible oxidation process and some other which undergo irreversible oxidation.

At low concentration (AQ1) the anodic peak potential decreased with increase in scan rate **(Table 1).** At higher concentrations the anodic peak potential increased with increase in scan rates **(Table 2-5) (Figure 2).** The cathodic peak potential Ec increases with increase (becomes less negative) in scan rate up to three concentrations (AQ1, AQ2, AQ3) and after that in decreases for AQ4 and AQ5.

Table 1: Cyclic peak parameters obtained for the aqueous extract AQ1 of *Pisonia grandis* at different scan rates at room temperature

Scan rate mV/s	Ea V	Ia 10^{-4} Amp/cm²	Ec V	Ic 10^{-4} Amp/cm²
10	0.4186	2.5153	0.6030	2.6328
20	0.4092	5.7266	0.5837	5.0381
50	0.3303	11.672	0.5743	8.9406
100	0.3268	15.412	0.5936	15.251
120	0.2769	26.872	0.4933	62.462

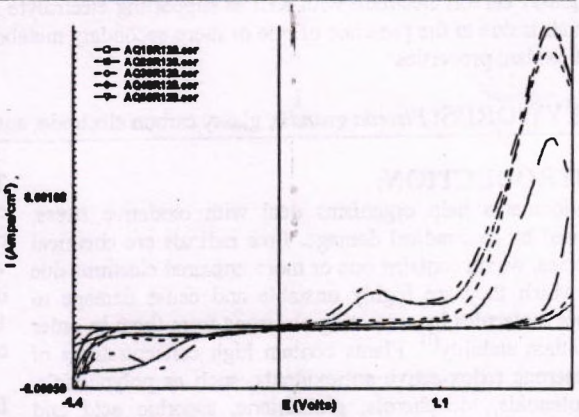
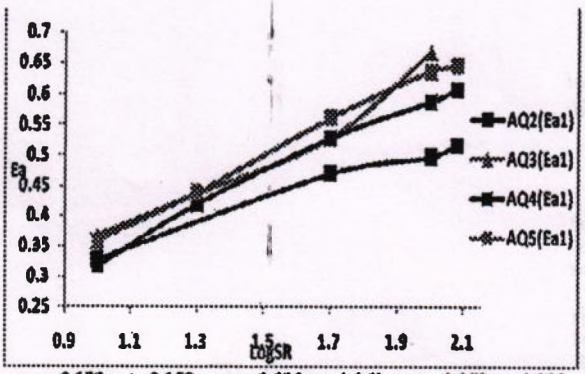


Figure 2: Cyclic voltammogram obtained for the aqueous extracts of *Pisonia grandis* at different concentration at room temperature

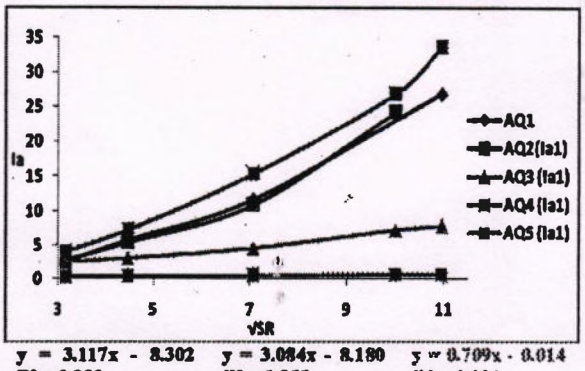
The anodic peak current (Ia) increases with increase in scan rates **(Figure 3).** This is because the current is directly proportional to the rate of electrolysis at the electrode surface. Electrolysis occurs at the electrode surface in response to a change in potential in order to maintain the surface concentrations of the oxidized and reduced species at the values required by the Nernst equation. Therefore, the faster the rate of change of potential (i.e., the scan rate), the faster the rate of electrolysis, and hence larger the current [4]. The cathodic peak current (Ic) decreases (more negative) with increase in scan rates. This electrochemical behavior may be attributed to the presence of one or more secondary metabolites having antioxidant properties like flavonoids, phenols and tri-terpenoids. This is quite evident from the

phytochemical colour tests of the extracts. The preliminary color tests showed the presence of flavonoids, phenols, tannins, saponins and tri- terpenoids in the aqueous fractionate of ethanol extract.



y = 0.173x + 0.158 y = 0.290x + 0.063 y = 0.273x + 0.085
R² = 0.983 R² = 0.968 R² = 0.997
y = 0.262x + 0.066
R² = 0.991

Figure3: Effect on anodic (Ea) potential of aqueous extract of *Pisonia grandis* at various scan rates and concentrations



y = 3.117x - 8.302 y = 3.084x - 8.180 y = 0.709x - 0.014
R² = 0.982 R² = 0.958 R² = 0.984
y = 3.711x - 8.991 y = 0.836x + 0.266
R² = 0.983 R² = 0.963

Figure 4: Effect on anodic (Ia) current of aqueous extract of *Pisonia grandis* at various scan rates and concentrations

Effect of concentration:

The concentration was increased by adding 1 ml (~40 mg/ml) of the sample. Phosphate buffer was added to adjust the pH to neutral. The voltammograms were studied for five different concentrations (AQ1), (AQ2), (AQ3), (AQ4), (AQ5) of the sample prepared.

Increase in concentration resulted in increase in electroactive species in solution. Anodic peak current (Ia), cathodic peak current (Ic) increases with increase in concentration, as the peak current is proportional to the concentration of electroactive species in the solution. But there is no much change in the anodic and cathodic peak potentials with concentration (Figure 2)

The anodic current may correspond to the concentration of antioxidants. The potential at the maximum of anodic wave reflects the reducing ability of antioxidants present [5]. For high concentration at high scan rate the anodic currents obtained were 29.25 (10^{-5}Amp) (Ia1), 247.5 (10^{-5} Amp) (Ia2) (Table 1-5).

Table 2: Cyclic peak parameters obtained for the aqueous extract AQ2 of *Pisonia grandis* at different scan rates at room temperature

Scan rate mV/s	Ea V		Ia 10^{-4} Amp/c²		Ec V	Ic 10^{-5} Amp/cm²
10	Ea1	0.3267	Ia1	2.8734	0.9513	4.1814
	Ea2	1.7197	Ia2	519.62		
20	Ea1	0.4577	Ia1	5.4315	0.8930	7.9065
	Ea2	1.7511	Ia2	734.29		
50	Ea1	0.4690	Ia1	10.947	0.7347	11.767
	Ea2	1.8029	Ia2	842.27		
100	Ea1	0.4961	Ia1	24.238	0.6521	30.442
	Ea2	1.8439	Ia2	1346.3		
120	Ea1	0.5165	Ia1	46.232	0.5424	49.343
	Ea2	1.8629	Ia2	1572.6		

Table 3: Cyclic peak parameters obtained for the aqueous extract AQ3 of *Pisonia grandis* at different scan rates at room temperature

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm²		Ec V	Ic 10^{-5} Amp/cm²
10	Ea1	0.3669	Ia1	2.546	0.8031	3.4722
	Ea2	1.6709	Ia2	124.44		
20	Ea1	0.4366	Ia1	3.024	0.8028	3.6577
	Ea2	1.6814	Ia2	141.66		
50	Ea1	0.5270	Ia1	4.5592	0.7336	6.2242
	Ea2	1.7773	Ia2	215.78		
100	Ea1	0.6675	Ia1	7.2369	0.7638	9.2822
	Ea2	1.8167	Ia2	215.08		
120	Ea1	0.9778	Ia1	7.8719	0.7640	10.131
	Ea2	1.8467	Ia2	228.3		

Table 4: Cyclic peak parameters obtained for the aqueous extract AQ4 of *Pisonia grandis* at different scan rates at room temperature

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm²		Ec V	Ic 10^{-5} Amp/cm²
10	Ea1	0.3172	Ia1	4.0363	0.6832	4.356
	Ea2	1.6519	Ia2	131.58		
20	Ea1	0.4183	Ia1	7.344	0.7435	6.6918
	Ea2	1.6898	Ia2	149.23		
50	Ea1	0.5271	Ia1	15.387	0.7630	14.881
	Ea2	1.7679	Ia2	188.16		
100	Ea1	0.5861	Ia1	26.926	0.8516	31.828
	Ea2	1.8379	Ia2	264.77		
120	Ea1	0.6061	Ia1	33.689	0.8117	33.259
	Ea2	1.8476	Ia2	254.56		

Table 5: Cyclic peak parameters obtained for the aqueous extract AQ5 of *Pisonia grandis* at different scan rates at room temperature

Scan rate mV/s	Ea V		Ia 10^{-5} Amp/cm²		Ec V	Ic 10^{-5} Amp/cm²
10	Ea1	0.358	Ia1	5.2264	0.7334	6.6278
	Ea2	1.7015	Ia2	113.75		
20	Ea1	0.4383	Ia1	6.9847	0.7637	6.9122
	Ea2	1.7211	Ia2	108.63		
50	Ea1	0.5601	Ia1	13.519	0.8152	13.98
	Ea2	1.7789	Ia2	153.02		
100	Ea1	0.6361	Ia1	22.494	0.8513	24.865
	Ea2	1.8176	Ia2	207.88		
120	Ea1	0.6461	Ia1	29.25	0.9906	32.626
	Ea2	1.8474	Ia2	247.5		

CONCLUSION:

Antioxidant activity of the aqueous fraction of ethanol extract was analyzed using cyclic voltammetry by varying concentrations and scan rates. Aqueous fraction of ethanol extract of *Pisonia grandis* showed significant antioxidant activity. The aqueous fraction gave two anodic peaks and one cathodic peak. The complimentary anodic and cathodic peaks reveal the redox reaction of the secondary metabolites present in the extract. The irreversible cathodic peak reveals the oxidation of one of the components in the extract. The antioxidant activity of extracts is quite obvious from the results.

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