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Cyclic voltammetric assessment of the antioxidant activity of petroleum ether extract of *Samanea saman* (Jacq.)Merr

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ABSTRACT

The Petroleum ether extract of Samanea saman (Jacq.)Merr. was investigated for its Phytochemical constituents. Cyclic voltammetric technique was used to assess its antioxidant activity. The results show the good antioxidant activity of the extract and the electrochemical behaviour of the constituents in it at glassy carbon electrode.

Keywords: *Samanea saman*, Antioxidant, cyclic voltammetry, glassy carbon.

INTRODUCTION

Samanea saman (Jacq.) Merr. Is distributed in the tropics and generally called as rain tree. It is cultivated as an ornamental shade tree, its pots and leaves are valued as cattle fodder. Rain tree is a folk remedy for cold, diarrhoea, headache, intestinal ailments and stomachache [1]. A multitude of minor uses is documented for *Samanea saman*, most of them of purely local significance, but all could be explored for wider applicability [2]. *Saman* and its products are being used as a source of medicine since long.

Among the most important constitutions of edible saman products, antioxidants are the most important species [3]. Antioxidants play a major role in protecting biological systems against many diseases. Over the last few years, reasonable supplementations of antioxidants have been widely practiced in different fields of industry and medicine to prevent and delay oxidative stress. Antioxidants are also used in dermocosmetic formulations in order to fight against oxidative stress which causes irreversible damages on skin, like psoriasis, cancer, premature aging [4]. The present paper is aimed at studying the electrochemical behaviour of the Petroleum ether extract of *Samanea saman* (Jacq.)Merr. By cyclic voltammetric technique and hence the assessment of its antioxidant activity from the anodic peak values.

MATERIALS AND METHODS

Collection of plant material
Combination of dried fallen leaves, flowers and stems of *Samanea saman* (Jacq.) Merr Collected from Coimbatore and it was used for the preparation of solvent extracts

Phytochemical analysis of petroleum ether extract of *Samanea saman*

Test for Alkaloids [5]: **Mayer's test**: A fraction of the extract was treated with Mayer's test reagent [1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water] and observed for the formation of cream coloured precipitate.
Wagner's test: A fraction of the extract was treated with Wagner's reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml water] and observed for the formation of reddish brown colour precipitate.

Test for Flavonoids [6, 7]**: Sulfuric acid test:** A fraction of the extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.

Shinoda test: About 0.5 of each extract portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

Test for Saponin [6, 8, 9]**: Foam test:** A small amount of extract was shaken with water looked for a persistent foam is formed

Test for Quinone [8]: A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Phenols [8]: **Ferric chloride Test:** The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

Liebermann's test: The extracts was heated with sodium nitrite, add H_2SO_4 solution diluted with water and add excess of dilute NaOH and observed for the formation of deep red or green or blue colour.

Test for Glycosides [5]: **Legal's test**: Dissolved the extract (0.1g) in pyridine, added sodium nitro prusside reagent and made alkaline with NaOH solution. Pink to red colour solution indicates the presence of glycosides.

Borntrager's test: The extract is hydrolyzed with concentrated HCl for 2 hours on a water bath and filtered and few ml of above filtrate was shaken with chloroform, chloroform layer was separated and added 10% ammonia, formation of pink colour indicates the presence of glycosides.

Antioxidant testing of *Samanea saman* extracts by cyclic votammogram

Instrumentation

The experimental set up for CV measurement consisted of a Solartron model number 1284 ZT electrochemical system (1280 B + USB 128087S) – CIF analyzer controlled by a personal computer with the Correware program. The calculations were performed with Corrview as user friendly computer interface.

Reagents and materials

Acetonitrile, acetic acid , 1litre 1M phosphate buffer solution (177.9g of disodium hydrogen phosphate (LR), 174g of dipotassium hydrogen phosphate (LR), 136g of potassium dihydrogen phosphate and 53.4g of ammonium chloride dissolved in 1000ml of distilled water), and 1M potassium chloride. De-ionized water was used for the preparation of all solutions.

Electrochemical measurements

Cyclic voltammetric experiments were performed using a three electrode system consisting of 3 diameter glassy carbon (MF 2012) as a working electrode, saturated calomel as a reference electrode and a platinum counter electrode also used as a reference. The response of a species at an electrode surface is strongly dependent on how the electrode has been prepared prior to running the experiment. Typically electrodes are polished and rinsed before the start of the experiment. The pretreatment of glassy carbon was according to reported procedures [10]. The mechanically pretreated electrode was then electrochemically pretreated by potentiodynamic cycling between 11.0 V to 0.8V in the supporting electrolyte at slow sweep rate of 10mV/s for 15-30 minutes. Deaeration of the electrolyte was continued during the potential cycling.

General procedure

The GCE was cleaned as in the procedure before each set of excrement's. In each study a blank run of the solvent and KCl supporting electrode were recorded to ascertain the wave due to the plant extracts and also ascertain the wave due to experiment. A low value of background current (10^{-6} / 10^{-7}) was found to be satisfactory for further studies.

About 20 ml of supporting electrode solution was dispensed into an electro chemical cell. To it was an added appropriate volume of phosphate buffer and plant extracts in a suitable solvent. The total volume was maintained to be 30ml using the respective solvents. The solution was stirred well for a minute using a magnetic stirrer. Cyclic Voltammetric measurements were run from +2V to -2V at different scan rate and at different concentration pH the plant extract at ~pH 6-7 at a glassy carbon electrode.

Variation of scan rate

For each of the CV runs made, the scan rate was varied as 10, 20, 50, 100 and 120 mV/scan, at different concentration of plant extracts (PE) and at room temperature at pH~ 6-7 in the presence of KCl as a supporting electrode.

Variation of concentration of plant extract

Five different concentrations were prepared by pipetting out 2, 3 and 4 ml of the stock solutions to get working solutions of ~ 10^{-5}M For each of the concentrations variation in ~pH 6-7 and scan rate was carried, in the presence of KCl as a supporting electrode and at room temperature.

Procedures for sample preparation for cyclic voltammetric experiments

20ml of 20:15:15 (de-ionized water: acetonitrile: acetic acid) mixture was added in a 1g of sample and allowed to refluxing for 3hrs in a heating mantle, filtered and preserved in a refrigerator, for further use. 5ml of KCl was added in an above 2ml (C1) filtrate, pH was measured, if a pH is less than seven, phosphate buffer was added to adjust the pH and the cyclic voltammogram were recorded. In the same way voltammograms were recorded for different concentration of the plant extract **(Table 1)**. From the cyclic voltammograms the following data were collected: Epa - anodic potential, Epc – cathodic potential, Ipa – anodic current, Ipc – cathodic current.

Table 1

Extract	Concentration(mg/ml)	Working pH
PE	C1(40)	6.63
	C2(80)	6.61
	C3(120)	6.61

RESULTS AND DISCUSSION

Phytochemical Screening of petroleum ether extracts of *Samanea Saman*

The petroleum ether (PE) extract showed the presence of alkaloids, flavonoids, saponins, quinine, phenol and glycosides.

Cyclic Voltammetric Behavior of *Samanea Saman* Extracts

Effect of Scan Rate on the Peak Current of *Samanea Saman* Extracts

Effects of scan rate on the peak current of all the extracts of *Samanea saman* were studied at GCE. The cyclic voltammograms of *Samanea saman* extract obtained for different concentrations at scan rates 10, 20 50, 100 and 120 are given in **Table 2-4.** In all the cases it is observed that the cathodic peak potential Epc value shift towards more negative side as the sweep rate υ increases. But the anodic peak potential shift towards more positive. The reversible peaks are shown in the **figure 1-4.** It is well evident from the results that the cathodic current decreases and anodic current increases with increasing sweep rate. Petroleum ether extracts of all the scan rate shows reversible process.

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 the larger the current .Antioxidant can be oxidized at an electrode and the more powerful the reducing agent, the lower is its positive oxidation potential[11].

In all the extracts of *Samanea saman* as shown in **figure 1-3** of anodic and cathodic current and potential of all the curves increases with increasing scan rate, it is directly proportional to the root of scan rate. The electro chemical behavior of the PE extract, well evident from the results might be attributed to the low molar weight antioxidant substances like flavonoids, phenolic etc present is it. This is obvious from the Phytochemical screening of the PE extract of *Samanea saman*.

Effect of Concentration on the Peak Current of *Samanea Saman* Extracts

The concentration of electro active species present in a solution also plays a major role in determining the response observed in a voltammetric experiment. The effect of concentration on the peak current (**figure 4**) of all extracts under study has been investigated for three different concentrations (2ml, 3ml, and 4ml) at varying scan rate 10, 20 50, 100 and 120 mV/s^{-1} at GCE.

The oxidation current for petroleum extract increased upon increasing extract concentration and reduction current decreased upon increasing concentration. Similarly the anodic potential increased and cathodic potential decreased with increasing concentration. In the **figure 4** Show liner relationship between the peaks current with the square root of scan rate. This indicated the process to be diffusion controlled.

The current corresponding to the reduction process increased as the concentration of the electro active species in the solution increased (**Table 2-4**). This indicates that the availability of active species at the electrode surface increases as the concentration of the electro active species increases [12].

Table 2: Cyclic Peak Parameters Obtained For the Petroleum Ether Extract PEC1 at Different Scan Rates

SR **mV/s^{-1}**	Ea	Ia × 10^{-6} **Amp/cm^2**	Ec	Ic × 10^{-6} **Amp/cm^2**	**Ec/Ea**	**Ic/Ia**
10	0.2592	0.5100	0.3439	0.2439	-1.32	-0.47
20	0.3400	0.8331	0.4143	0.5120	-1.21	-0.61
50	0.4682	1.6435	0.5559	1.3063	-1.18	-0.79
100	0.6285	2.6728	0.7312	1.9765	-1.16	-0.73
120	0.6384	2.9706	0.8313	3.7305	-1.3	-1.25

Table 3: Cyclic Peak Parameters Obtained For the Petroleum Ether Extract PEC2 at Different Scan Rates

SR **mV/s^{-1}**	Ea	Ia × 10^{-5} **Amp/cm^2**	Ec	Ic × 10^{-5} **Amp/cm^2**	**Ec/Ea**	**Ic/Ia**
10	0.2388	0.4646	0.1846	0.2735	-0.77	-0.58
20	0.3702	0.8046	0.2351	0.4725	-0.63	-0.58
50	0.4781	1.7103	0.4259	1.0172	-0.89	-0.59
100	0.5783	2.7593	0.5817	2.0491	-1.00	-0.74
120	0.6887	3.2898	0.7311	3.5229	-1.06	-1.07

Table 4: Cyclic Peak Parameters Obtained For the Petroleum Ether Extract PEC3 at Different Scan Rates

SR **mV/s^{-1}**	Ea	Ia × 10^{-5} **Amp/cm^2**	Ec	Ic × 10^{-5} **Amp/cm^2**	**Ec/Ea**	**Ic/Ia**
10	0.2391	0.4692	0.22447	0.23381	-0.93	-0.49
20	0.3805	0.8537	0.3643	0.4532	-0.95	-0.53
50	0.5079	1.7611	0.4754	1.0566	-0.93	-0.58
100	0.6884	3.4660	0.5619	2.0194	-0.81	-0.58
120	0.7085	3.4985	0.8809	2.5619	-1.24	-0.61

Figure 1: Cyclic Voltammogram Of Petroleum Ether Extract (PEC1) Of *Samanea saman* At Various Scan Rates

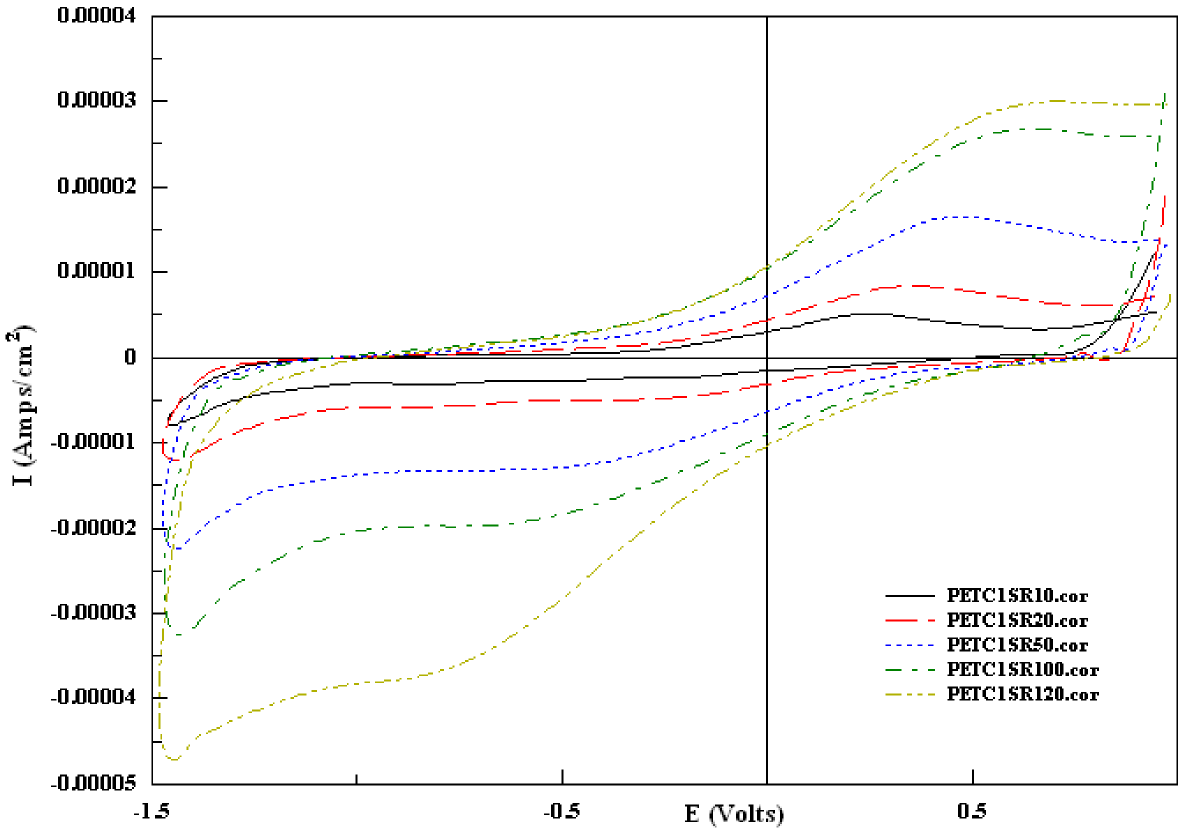


Figure 2: Cyclic Voltammogram Of Petroleum Ether Extract (PEC2) Of *Samanea saman* At Various Scan Rates

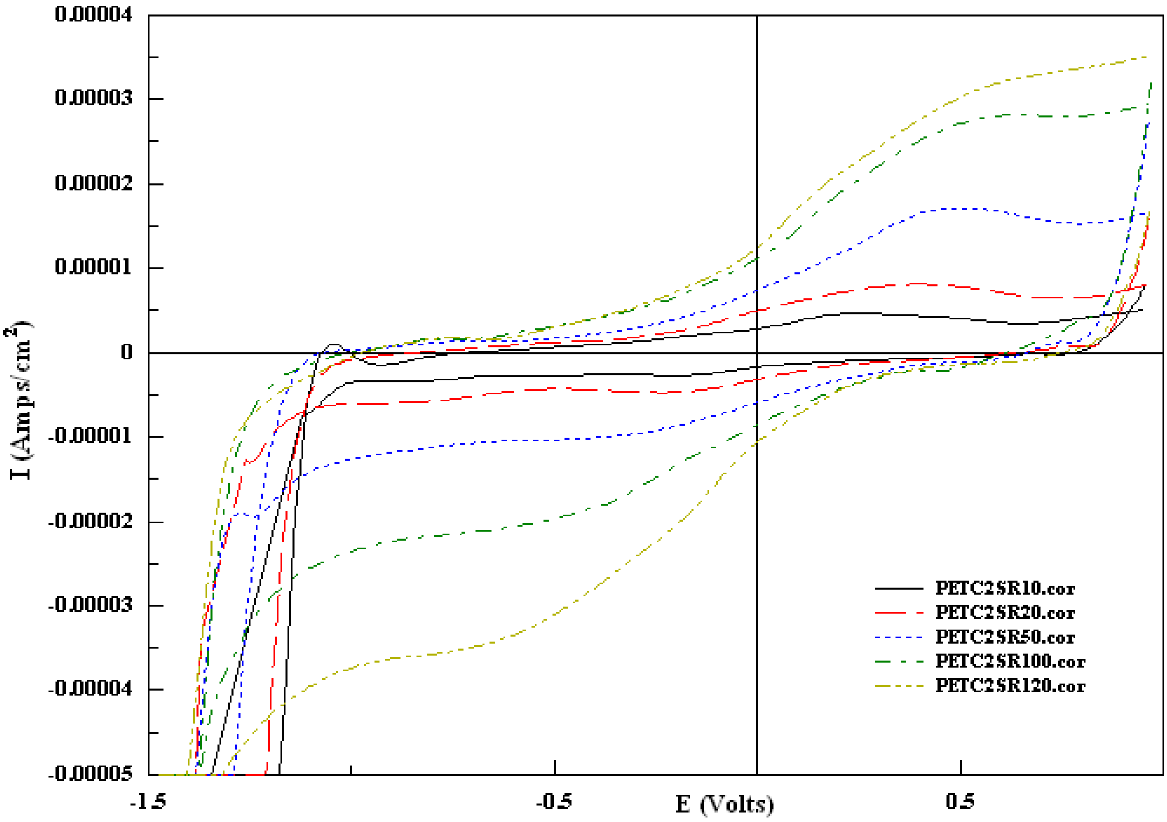


Figure3: Cyclic Voltammogram Of Petroleum Ether Extract (PEC3) Of *Samanea saman* At Various Scan Rates

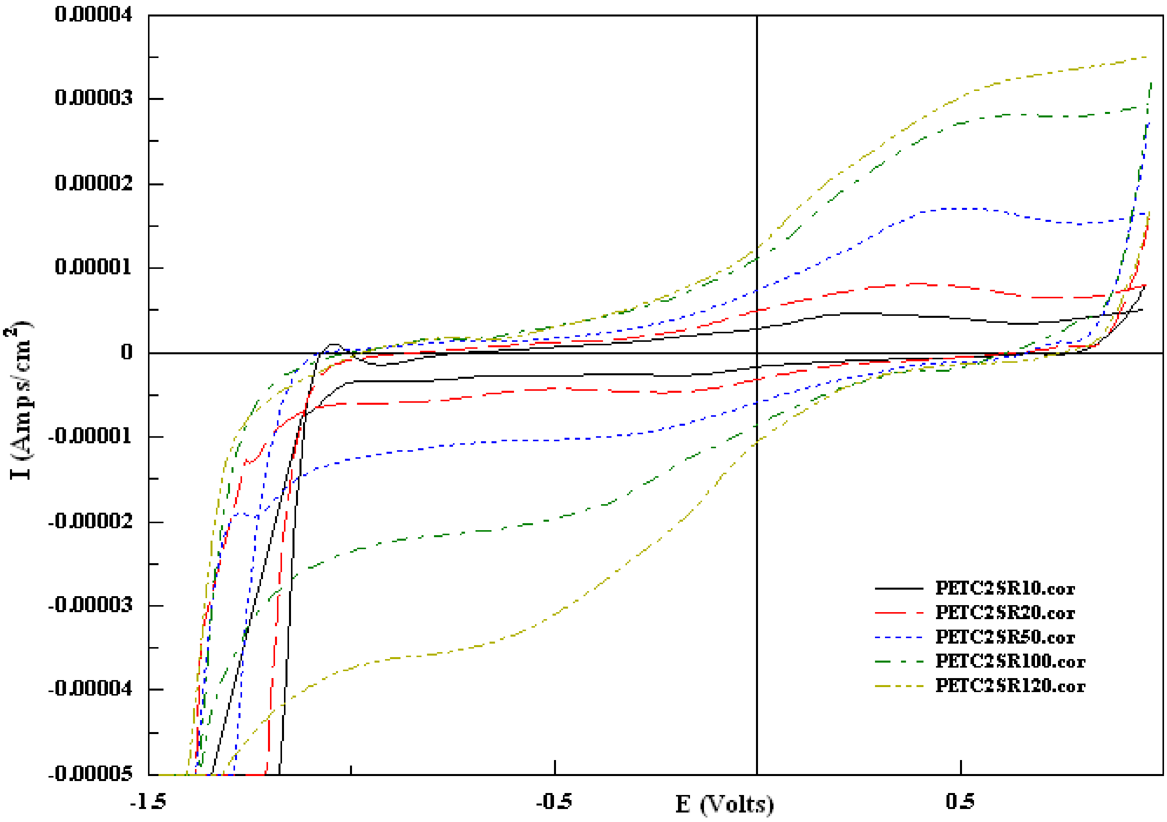


Figure 4:Cyclic Voltammogram Of Petroleum Ether Extract (SR 100) Of *Samanea saman* At Various Concentrations

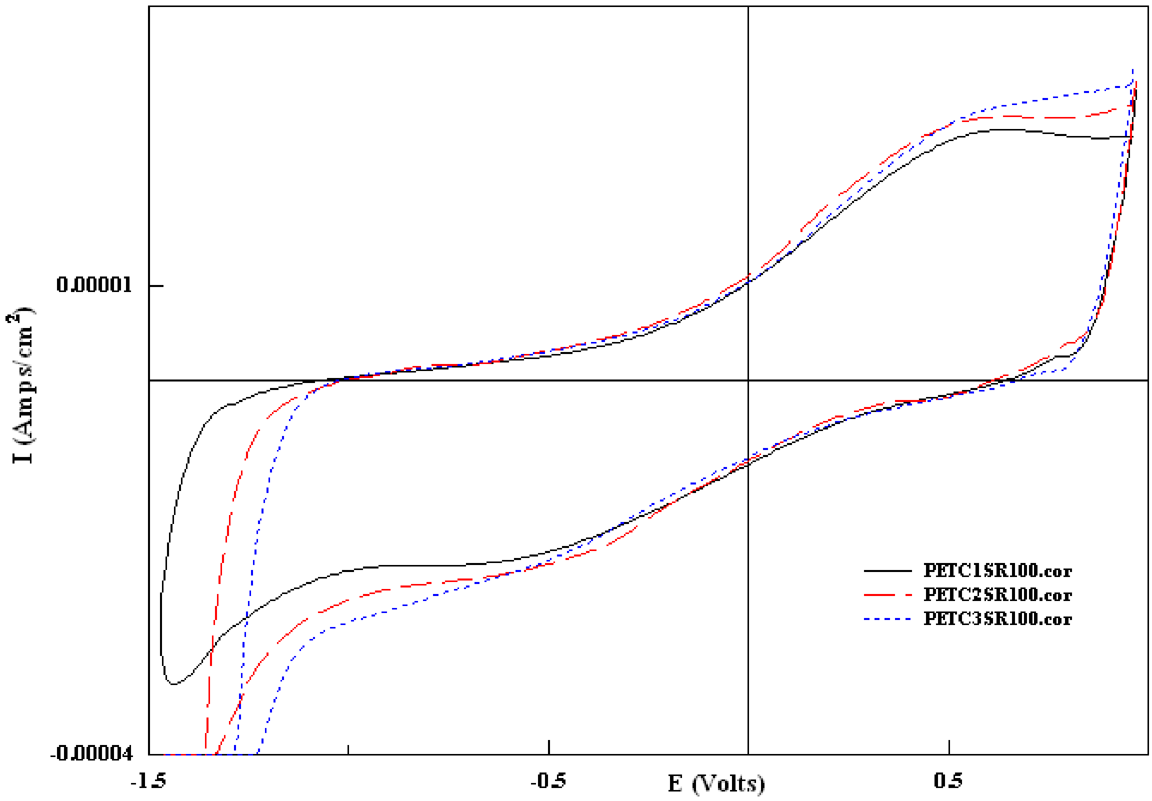
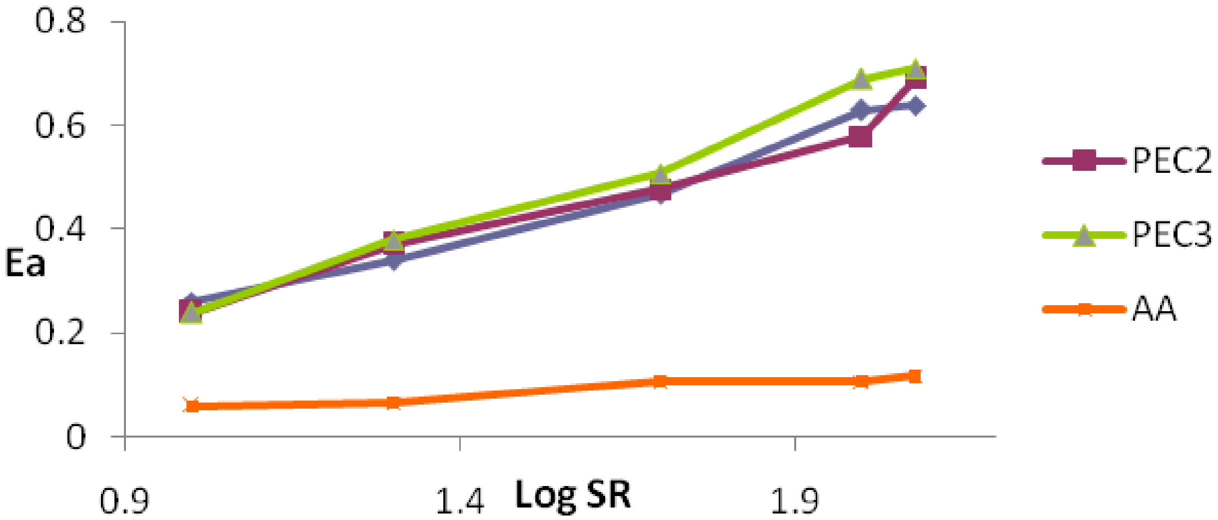
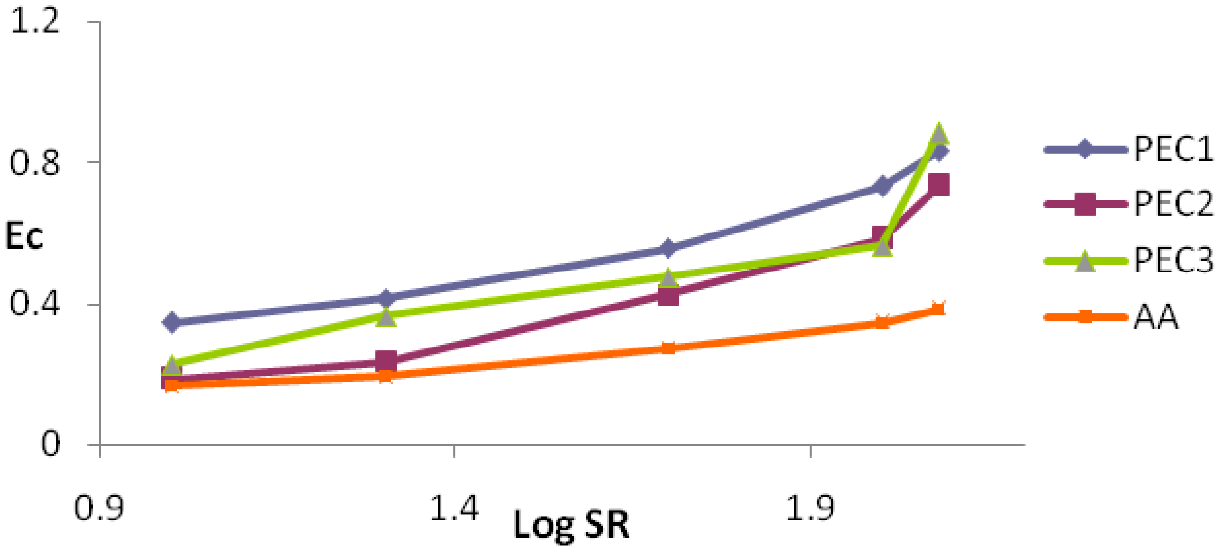


Figure 5: Effect On Anodic(Ea) And Cathodic(Ec) Peak Potential Of Petroleum Ether Extract Of *Samanea saman* At Various Scan Rates And Concentrations

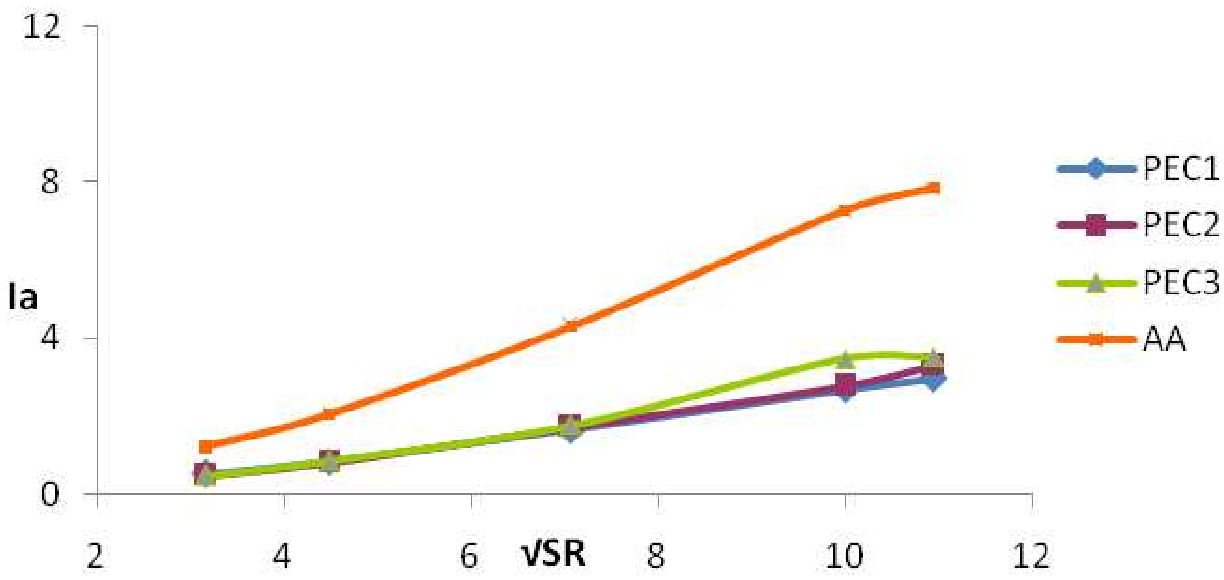


PEC1	PEC2	PEC3	AA
y = 0.374x - 0.134 R^2 = 0.966	y = 0.374x - 0.134 R^2 = 0.966	y = 0.434x - 0.196 R^2 = 0.990	y = 0.8822x - 1.7638 R^2 = 0.9967

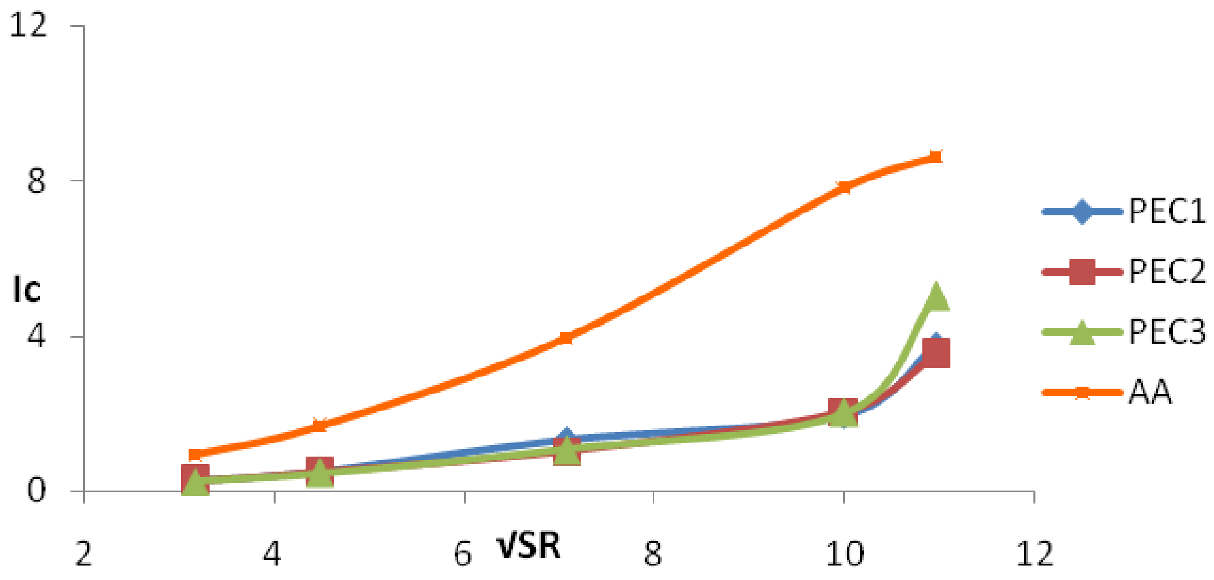


PEC1	PEC2	PEC3	AA
y = 0.436x - 0.130 R^2 = 0.952	y = 0.483x - 0.349 R^2 = 0.935	y = 0.484x - 0.281 R^2 = 0.815	y = 1.0313x - 2.7603 R^2 = 0.9872

Figure 6: Effect On Anodic(Ia) And Cathodic(Ic) Peak Current Of Petroleum Ether Extract Of *Samanea saman* At Various Scan Rates And Concentrations



PEC1	PEC2	PEC3	AA
y = 0.133x + 0.135 R^2 = 0.989	y = 0.099x + 0.233 R^2 = 0.984	y = 0.061x + 0.277 R^2 = 0.921	y = 0.8822x - 1.76 R^2 = 0.9967



PEC1	PEC2	PEC3	AA
y = 0.383x - 1.181 R^2 = 0.863	y = 0.370x - 1.177 R^2 = 0.874	y = 0.500x - 1.807 R^2 = 0.745	y = 1.0313x - 2.76 R^2 = 0.9872

CONCLUSION

The phytochemical screening procedures revealed the presence of important biologically active products in the dried fallen plant parts of *Samanea saman*, signifying its importance in phytochemistry. The cyclic voltammetric behaviors of petroleum ether extract show the presence of anodic and cathodic peak portraying the redox process of extracts. The rate of Ec and Ea was found to be unity in all cases showing the process of reversible mechanism operating in the redox process. The antioxidant properties of the extracts were assessed by cyclic voltammetric method from its oxidation potential values. Generally there is a relationship between antioxidative and per oxidative activities and oxidative potentials. The lower the antioxidant potential of extracts higher would be the antioxidant capacity. Lower the oxidative potential, higher is the ability to donate electron easily to the system generate free radicals.

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