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# Evaluation of antioxidant activity of ethyl acetate extract of *Samanea saman* (Jacq.)Merr by cyclic voltammetry

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## ABSTRACT

*The electrochemical behavior of ethyl acetate extract of Samanea saman (Jacq.)Merr were studied by cyclic voltammetry using a three electrode system. The extracts were investigated for presence of phytochemical constituents and antioxidant activity assessed from its oxidation potential values at glassy carbon electrode. A reproducibility of oxidation potential, height of peaks and calibration slops were obtained.*

**Keywords:** *Samanea saman*, Antioxidant determination, cyclic voltammetry.

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## INTRODUCTION

Active oxygen species and free radicals play an important role in the initiation and evolution of numerous diseases [1]. The use of compounds with antioxidant activity is expected to be useful for the treatment of these diseases [1]. Therefore, has been a growing interest in finding novel antioxidants in order to meet the requirements of pharmaceutical industries.

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. It offers a rapid location of redox potentials of the electroactive species [2]. Voltammetry is a convenient methodology for the study of antioxidant properties and the determination of antioxidant activity of biological systems.

*Samanea saman* (Jacq.) Merr is distributed in the tropics and generally called as rain tree. A multitude of minor uses is documented for rain tree, most of them of purely local significance, but all could be explored for wider applicability [3].

## MATERIALS AND METHODS

**Collection of plant material**

Combination of dried fallen parts likes leaves, flowers and stems of *Samanea saman* (Jacq.) Merr collected from Coimbatore was used for the preparation of solvent extracts.

**Phytochemical analysis of ethyl acetate extract of *Samanea saman***

**Test for Alkaloids** [4]: **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** [5, 6]**: 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 coloration indicates the presence of flavonoids.

**Test for Tannin** [5,7]: The plant extract was dissolved in water, it was heated on a water hath for 1 hour and the filtrate was treated with ferric chloride and observed for the formation of dark green colour.

**Braymer's test:** One ml of extract was treated with 10% alcoholic ferric chloride solution was added and observed for the formation of blue or greenish grey colour solution.

**Test for Saponin** [5, 7, 8]**: Foam test:** A small amount of extract was shaken with water looked for a persistent foam is formed.

**Test for Quinone** [7]: A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

**Test for Phenols** [7]: **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 Anthocyanin** [4]: The extract of alcohol was treated with HCl and observed for the same colour to indicate the presence of anthocyanin. The disappearance of the colour indicates the presence of beta cyanine.

**Test for Glycosides** [4]: **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 voltammetry**

**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.

**Electrochemical measurements**

Cyclic voltammetric experiments were performed using a three electrode system consisting of three 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 [9]. 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.

**General procedure**

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 ethyl acetate extract 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

**Table 1**

| Extract | Concentration(mg/ml) | Working pH |
|---|---|---|
| EA | C1(40) | 6.54 |
| | C2(80) | 6.45 |
| | C3(120) | 6.28 |

*voltammograms the following data were collected: Epa - anodic potential, Epc – cathodic potential, Ipa – anodic current, Ipc – cathodic current.*

## RESULTS AND DISCUSSION

**Phytochemical Screening of petroleum ether extracts of *Samanea Saman***

The ethyl acetate (EA) extract showed the presence of alkaloids, flavonoids, tannin, saponins, quinine, phenol, betacyanin and glycosides.

**Cyclic Voltammetric Behavior of *Samanea Saman* Extracts**

**Effect of Scan Rate on the Peak Current of ethyl acetate extracts**

Effects of scan rate on the peak current of all the ethyl acetate extract of *Samanea saman* were studied at GCE. The cyclic voltammograms of ethyl acetate 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. Ethyl acetate extract of the entire scan rate shows reversible process. 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[10].

In all the ethyl acetate extract of *Samanea saman* as shown in figure 1-3 and 5 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 ethyl acetate 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 ethyl acetate 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 ethyl acetate extract 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 ethyl acetate 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. Figure 4 and 6 shows a linear relationship between the peaks current with the square root of scan rate. This indicates 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.

**Table 2: Cyclic Peak Parameters Obtained For the Ethyl Acetate Extract EAC1 at Different Scan Rates**

| SR **$mV/s^{-1}$** | Ea V | Ia $\times 10^{-5}$ **$Amp/cm^2$** | Ec V | Ic $\times 10^{-5}$ **$Amp/cm^2$** |
|---|---|---|---|---|
| 10 | 0.19906 | 0.63435 | 0.16533 | 0.31942 |
| 20 | 0.26063 | 1.1773 | 0.18561 | 0.58769 |
| 50 | 0.35796 | 2.5849 | 0.3172 | 1.3753 |
| 100 | 0.4483 | 4.5416 | 0.49255 | 2.5354 |
| 120 | 0.46814 | 5.3113 | 0.50232 | 3.3575 |

**Table 3: Cyclic Peak Parameters Obtained For the Ethyl Acetate Extract EAC2 at Different Scan Rates**

| SR **$mV/s^{-1}$** | Ea V | Ia $\times 10^{-5}$ **$Amp/cm^2$** | Ec V | Ic $\times 10^{-5}$ **$Amp/cm^2$** |
|---|---|---|---|---|
| 10 | 0.27929 | 0.61483 | 0.23475 | 0.31843 |
| 20 | 0.34092 | 1.1145 | 0.29519 | 0.58903 |
| 50 | 0.45828 | 2.2896 | 0.4828 | 1.6943 |
| 100 | 0.59859 | 3.7648 | 0.7716 | 4.3548 |
| 120 | 0.60685 | 4.5263 | 0.6815 | 3.2086 |

**Table 4: Cyclic Peak Parameters Obtained For the Ethyl Acetate Extract EAC3 at Different Scan Rates**

| SR **$mV/s^{-1}$** | Ea V | Ia $\times 10^{-5}$ **$Amp/cm^2$** | Ec V | Ic $\times 10^{-5}$ **$Amp/cm^2$** |
|---|---|---|---|---|
| 10 | 0.35972 | 0.59035 | 0.34395 | 0.2999 |
| 20 | 0.40136 | 0.10428 | 0.41436 | 0.5613 |
| 50 | 0.53862 | 1.9834 | 0.53577 | 1.2055 |
| 100 | 0.61885 | 3.1261 | 0.69137 | 2.2396 |
| 120 | 0.69895 | 3.6354 | 0.73104 | 3.0993 |

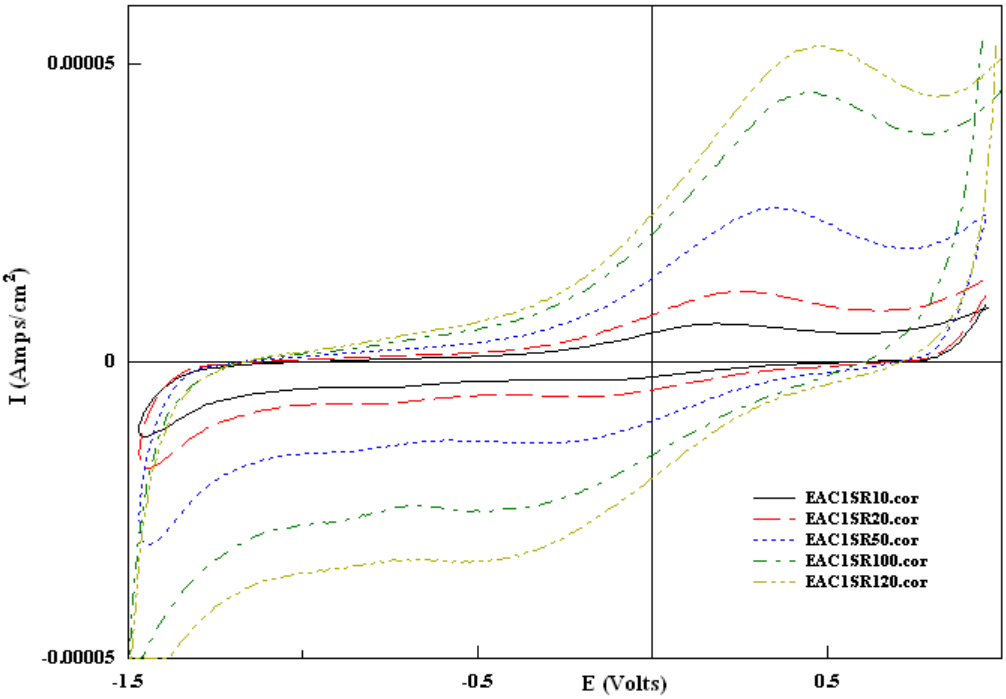



**Figure 1: Cyclic Voltammogram Of Ethyl Acetate Extract (EAC1) Of *Samanea saman* At Various Scan Rates**

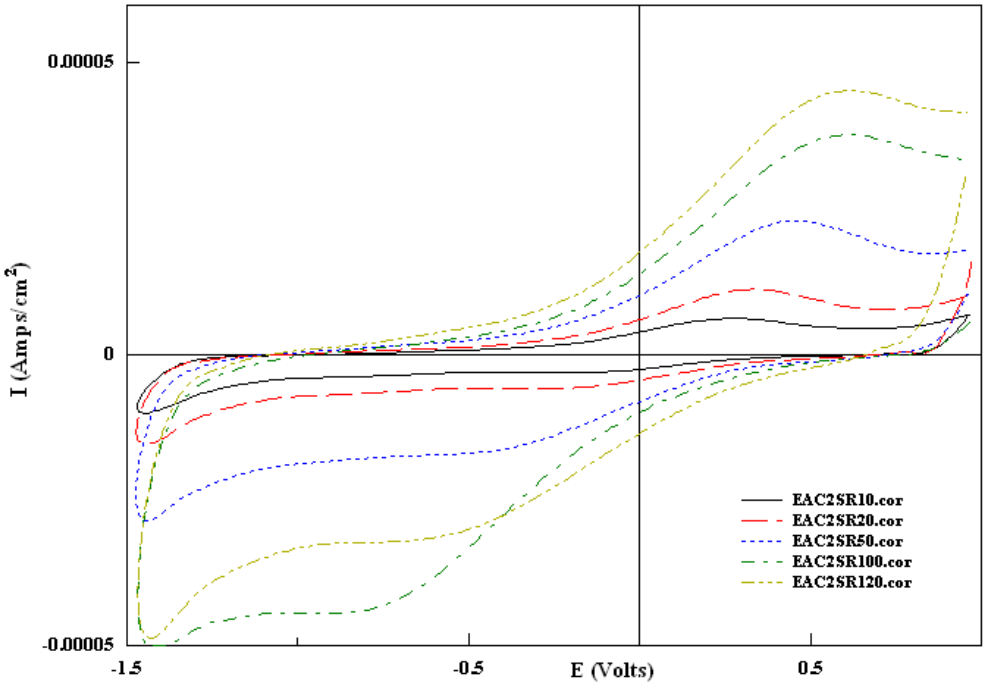



**Figure 2: Cyclic Voltammogram Of Ethyl Acetate Extract (EAC2) Of *Samanea saman* At Various Scan Rates**

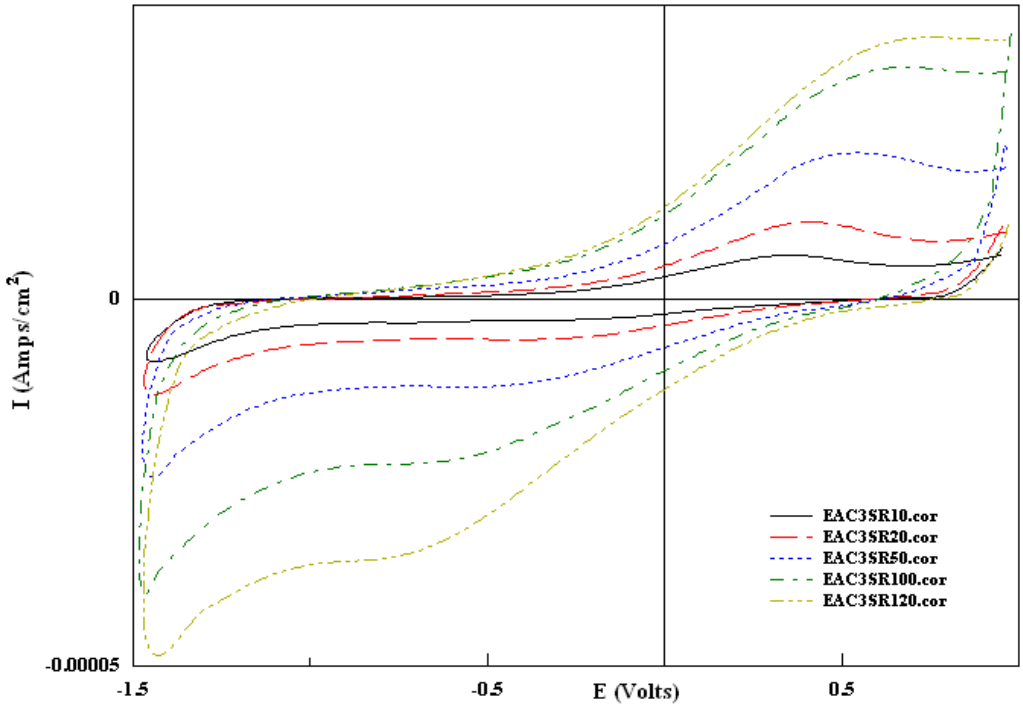



**Figure3: Cyclic Voltammogram Of Ethyl Acetate Extract (EAC3) Of *Samanea saman* At Various Scan Rates**

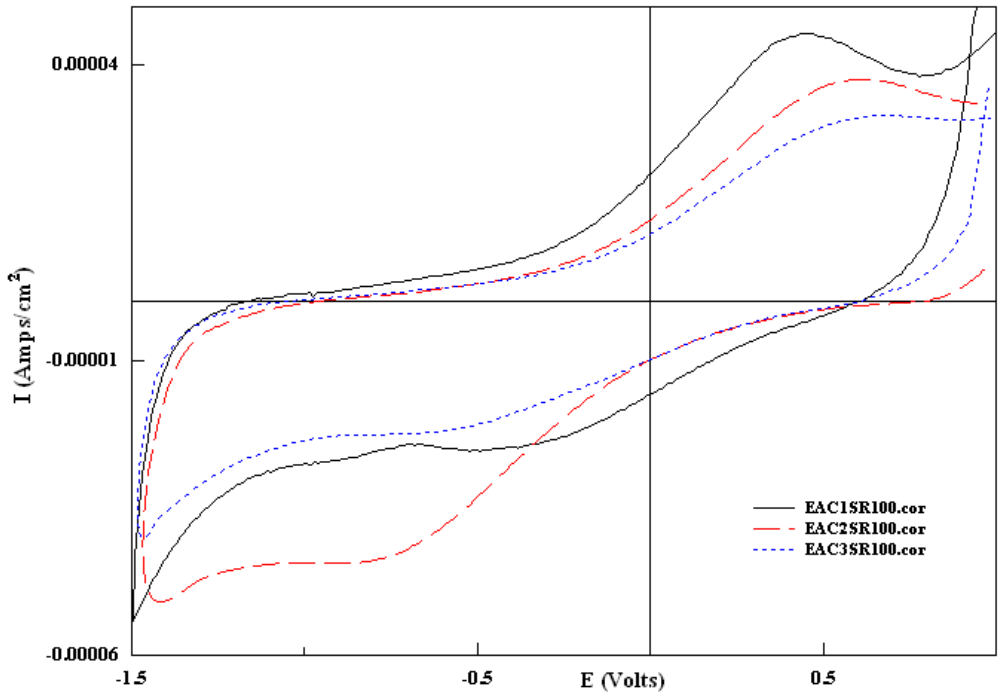
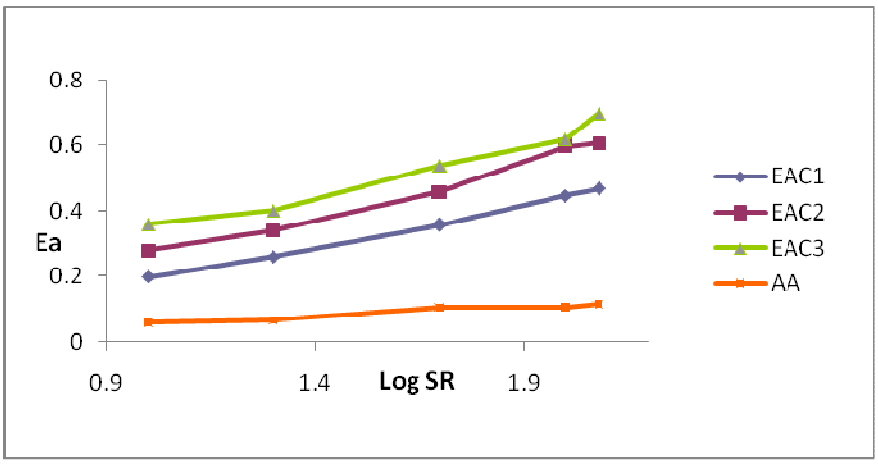
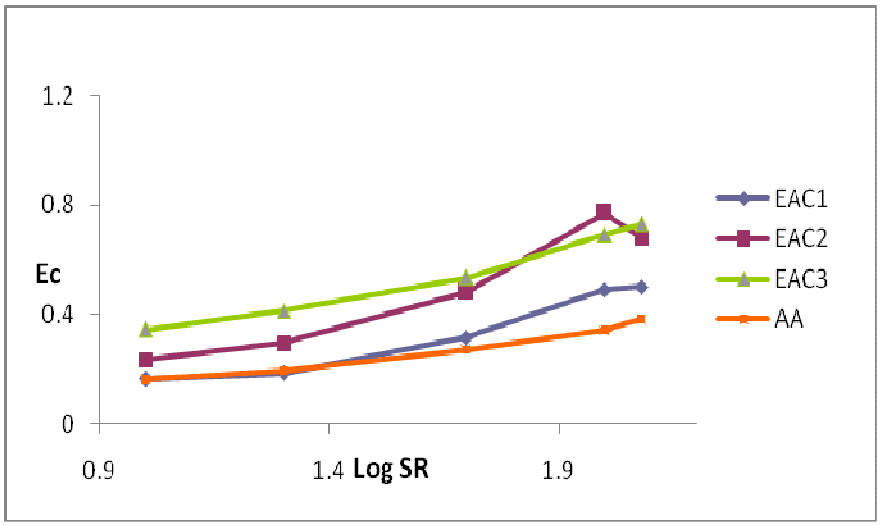



**Figure 4: Cyclic Voltammogram Of Ethyl Acetate Extract (SR 100) Of *Samanea saman* At Various Concentrations**

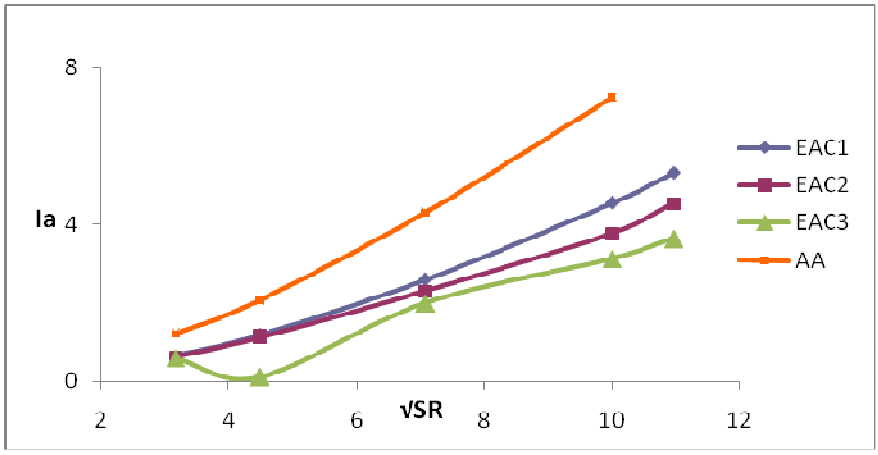



| EAC1 | EAC2 | EAC3 | AA |
|---|---|---|---|
| y = 0.252x - 0.061<br>$R^2$ = 0.995 | y = 0.318x - 0.057<br>$R^2$ = 0.981 | y = 0.305x + 0.030<br>$R^2$ = 0.963 | Y=0.8822x+1.763<br>$R^2$ = 0.9967 |

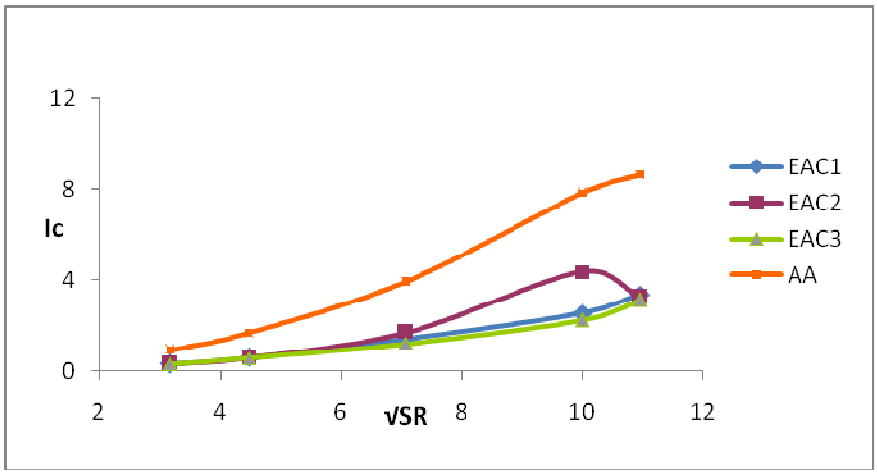



| EAC1 | EAC2 | EAC3 | AA |
|---|---|---|---|
| y = 0.339x - 0.216<br>$R^2$ = 0.939 | y = 0.489x - 0.297<br>$R^2$ = 0.926 | y = 0.361x - 0.041<br>$R^2$ = 0.976 | y = 1.0313x-2.76<br>$R^2$ = 0.9872 |

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




| EAC1 | EAC2 | EAC3 | AA |
|---|---|---|---|
| y = 0.602x - 1.447<br>$R^2$ = 0.993 | y = 0.493x - 1.059<br>$R^2$ = 0.993 | y = 0.440x - 1.254<br>$R^2$ = 0.936 | y = 0.8822x - 1.763<br>$R^2$ = 0.9967 |




| EAC1 | EAC2 | EAC3 | AA |
|---|---|---|---|
| y = 0.376x - 1.049<br>$R^2$ = 0.969 | y = 0.477x - 1.374<br>$R^2$ = 0.875 | y = 0.339x - 0.941<br>$R^2$ = 0.956 | y = 1.031x - 2.76<br>$R^2$ = 0.9872 |

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

## 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 ethyl acetate 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 ethyl acetate extract showed the less oxidation potential, lower the antioxidant potential of extract 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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