IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 15, Issue 7 Ser. I (July 2022), PP 15-23
www.iosrjournals.org

Redox Mode of SET/SHT in the determination of Antioxidant potential of Phyto Chemical compounds / Cu (II)Nc complex in Electrochemical work Station

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Abstract
Medicinal plants are playing vital role in the protection of the people from major ailments such cancer, Hepatitis, CNS disorders etc. Previous study reported thatcancer cells were induced by free radicals and were controlled by more number of mechanisms involved in medicinal chemistry. Among these (SHT) single proton transfer mode / single electron transfer modes (SET) are the important mechanisms to control the free radicals. These modes were involved in the determination of antioxidant ability of phyto compounds. By the release of H / e- while the scavenging activity of phytocompounds through SHT and SET modes, the free radicals were controlled from further chain reactions.The controlling ability determined the antioxidant potential of phytochemical compounds. In this research a Novel redoxprobe, Copper II Neocuproine was used in electrochemical work station and determined the antioxidant potentials of different parts of the extracts of medicinal plants such as Phyllanthus emblica (gooseberry leaves)Coriandrumsativum (seed), Piper nigrum (Pepper), Curcuma longa and Vetiveriazizanioides root (vetriver).Extracts were prepared by sonication and the Qualitative analysis showed the presence of carbohydrates, alkaloids, terpenoids, phenolic compounds, flavonoids, quinines, coumarins cardiac glycerides, amino acids, steroidal compounds etc.Literature study revealed that these compounds have shown medicinal applications. Lot of functional groups such as phenolic, ketone, aldehyde, acidic, amino, aromatic, conjugation groups easily released the electrons / protons and reduced the free radicals which caused cancer in our body.
The above mentioned phyto compounds acted as antioxidants and neutralized the free radicals by giving up some of their own electrons/ Protons hence "off" switch for the free radicals. The release of H/e – were measured in electrochemical work station and identified the (ΔE) reduction potential of the phytochemical extracts as Curcuma longa-0.337mV, Phyllanthus emblica-0.392MV , Coriandrumsativum- 0.483, Piper nigrum- 0.492 , Vetiveriazizanioides root- 0.532 for each 500μlof plant extract in 25ml of Copper complex. Lower the reduction potential value showed higher the antioxidant capacity. Among these Curcuma longa at 0.337mV and Phyllanthus emblica at 0.392mV were observed asthe best anti-oxidants.

Key words
Copper II Neocuproine, Medicinal plants, phyto compounds, Electro chemical workstation, anti-oxidant potential.

Date of Submission: 22-06-2022 Date of Acceptance: 04-07-2022

I. Introduction

Environment contributes a lot of resources to the well-being of the human. Air, water, soil and plants play a vital role in their health issues. Among these, medicinal plants are nature's gift to avail easy and cheap in their day today life. Plant extracts consist of numerous phyto chemicals and have antioxidant potential. Oxidative damage in our body plays a significant pathological role in human diseases. Free radicals lead to cellular necrosis, atherosclerosis, rheumatoid arthritis, affect the CNS, aging, respiratory diseases, liver diseases and cancer [2-4]. Natural plants consist of antioxidant compounds, and they protect the cell by its single H, single e- , single oxygen species, singlet oxygen, super oxide, hydroxyl radicals and peroxynitrite [5-7]. Previous study revealed the antioxidant properties of the medicinal plants and explained the bio applications of Phenolic compounds, quinone, alkaloid, flavonoid and Coumarin showed anti-oxidant potential approximately at 0.397V, 0.492V, 0.321, 0632 and 0.523v respectively [4-7] . This study aims to investigate the antioxidant potential of some important medicinal plants using a novel redox probe, Copper II neocuproine in electrochemical workstation and identified the best antioxidant among them by their redoxpotential.

II. Material And Methods

2.1 Extraction of Phytocompounds by Sonication

In this study different parts of the medicinal plants such as Phyllanthus emblica (gooseberry leaves) Coriandrumsativum (seed), Piper nigrum (Pepper), Curcuma longa and Vetiveriazizanioides root (vetriver) were collected and powdered. After collection they were processed soon to prevent the deterioration of secondary metabolites present in the sample. The extraction of phytochemical components from plants were derived from greener technique as Ultrasound sonication process in which a frequency range of > 20 KHz electrical sound energy transformed into physical vibration mode and acted on the surface of the chemical compounds. It agitates the solid plants at room temperature and disrupted the cellular chemical compounds into solution without damaging the original nature.

2.2 Synthesis of Copper II neocuproine complex

Copper(II) acetate monohydrate (0.100 g, 0.5 mmol) was dissolved in ethanol (15 mL) and a solution of neocuproine, (0.104 g, 0.5 mmol) in ethanol (15 mL) was added with continuous stirring and heating (60 °C). To this royal blue solution a clear solution of 5ml of Phosphate buffer was added. After 15 min of stirring, 10 mL of water were added. The solution was stirred for 20 min at 60 °C, filtered and allowed to cool. From this solution, green crystals as shown in figure - were obtained (yield 80%). Analysis found: C 54.71, H 3.83, N 5.96%; calculated: 54.79, H 3.72, N 6.08.

2.3 Analytical techniques-Electro chemical work station

The clear solution of plant extracts are diluted to1: 100 concentrations. Supporting electrolyte Copper II neocuproine was prepared in ethanol and calibration curves are constructed with ascorbic acid. Total antioxidant capacity is analysed by electrochemical methods used as Barros [2-4]. The cyclic voltammograms are obtained by using scan rate, at 25, 50, 100, pulse width, and pulse amplitude .These parameters were used by Barros et al. The peaks for total anodic and cathodic currents are determined by CV and calculate the antioxidant potential by reduction potential values. The voltammetry cell consists of three electrodes such as reference, working and supportive electrode. Cyclic voltammeter is a powerful technique to calculate the oxidation and reduction potential of chemical components. The information regarding the analyte was taken from the cyclic voltammograms. Anodic peak potential Epa, Cathodic peak potential Eca, Anodic current Ia and Cathodic current Ic which were measured using the peak parameters operation.

The peak potential separation DEp = Epc-Epa = 59.2 /n mV at all scan rates. The redox cyclic current voltammograms were shown in figure-2. In phytochemical extracts, important medicinal compounds such as cardiac glycoside, coumarins, quinine, anthraquinone, Phenolic compounds were identified. They undergo redox reactions and produce the electro voltammograms as shown in figure-2.The peak potential separation DEp (= Epc - Epa) = 59.2/n mV at all scan rates at 25 oC.The peak current ratio = ipa/ipc = 1 at all scan rates and the peak current function ip/n1/2 (n = scan rate) is independent of n.13

Ip= 2.69x105 n

3/2AD1/2 CV1/2

I-Peak Current potential,

n= number of electrons

A=Surface area, D=Diffusion coefficient, C=concentration of the electrolyte.

III. Results And Discussion

3.1Qualitative results of Phyto compounds

Phytochemical analysis of the extracts was carried out .They showed the positive results for the phyto chemical components such as Carbohydrates, amino acid, alkaloids terpenoids, cholesterol, flavonoids, ascorbic acid, quinone, coumarins etc., and the results were shown in the table 1. Alcoholic extract consisted more numbers than aqueous components. Among these chemical components, more number of compounds from all plants possessed OH group hence they could easily involve in the SHT mode during the redox reaction with Copper II neocuproine and the mode of mechanism was showe in figure- and acted as the Free Radical scavenger. Ascorbic acid and other polyphenol compounds, tannin, Gallic acid directly involved in SHT mode and involved in scavenging free radical. Structures of the analysed phyto compounds were showed in the figures 3.1 to 3.3. It confirmed the presence of one H atom of their own and involved in SHT mode.

Table-3.1Results for Qualitative analysis

Phyto chemical analysis in different solvents

Phyto chemical components	PE		CL	
	Aqueous extract	Alcoholic Extract	Aqueous extract	Alcoholic Extract
Carbohydrates	+	-	+	-
Amino acids	+	+	+	+
Terpenoid	-	+	+	+
Alkaloid	+	+	-	+
Flavonoid	-	+	+	+
Coumarin	+	+	+	+
Quinone	-	+		+
Phytosterol	-	+	-	+
Anthocyanin	+	+	+	+
Cholesterol	-	+	-	+
Polyphenol	+	+	+	+
Ascorbic acid	+	-	+	-

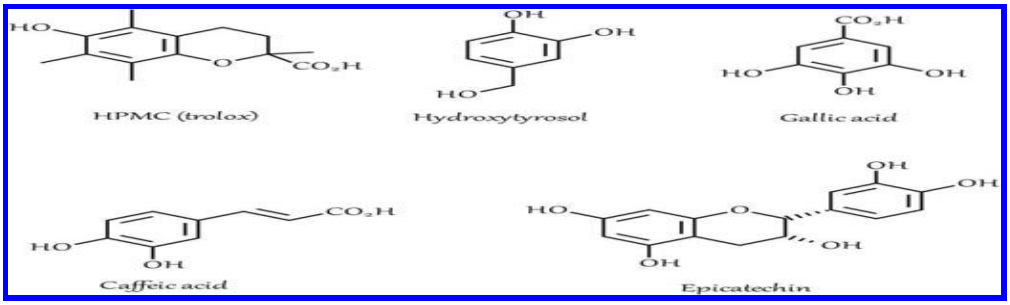


Figure -3.1 Phyto Compounds found in Plant extracts

Figure – 3.2 Phytocompounds found in *Curcuma longa,Phyllanthus emblica , Coriandrumsativum, Piper nigrum*

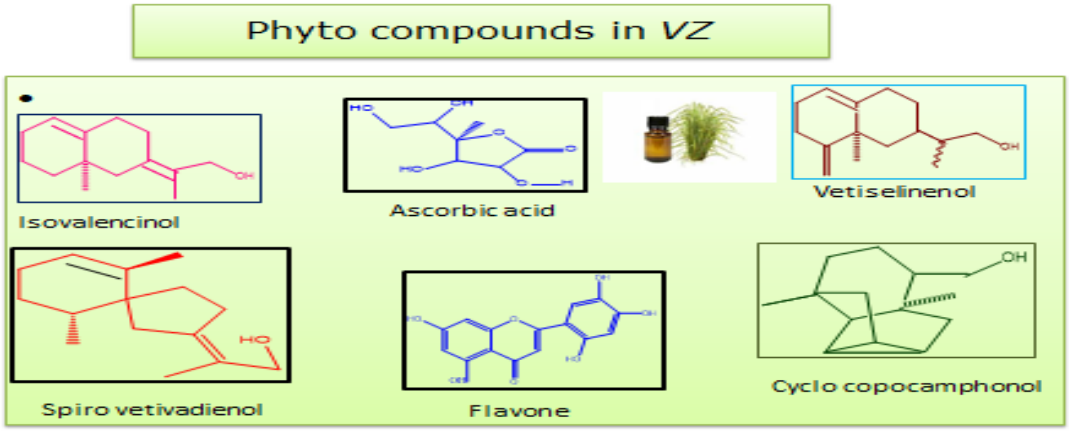


Figure –3.3Phytocompounds found in *Vetiveriazizanioides* root

3.2 Thin Layer Chromatographic analysis

All the plant extracts were subjected to thin layer chromatography to determine the number of phytocompound identified in the analysis. More number of compounds were identified with different R_f values. The R_f values have shown in the figure 3.4

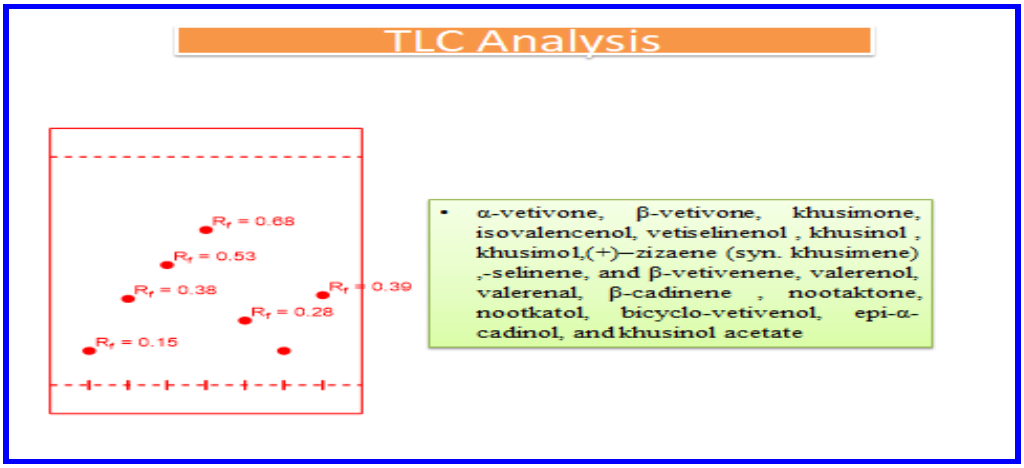


Figure-3.4R_f values for analysed phyto componds.

3.4 H^{13}NMR Predictions

Proton NMR spectrum predicted the structure of the Cu (II) and Ligand complex

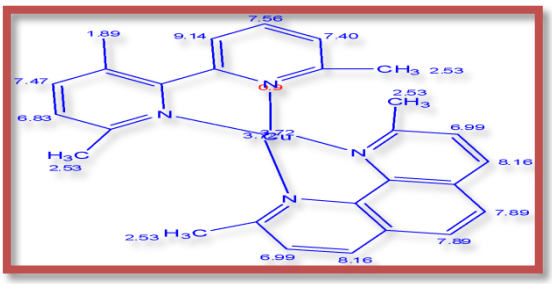


Figure- 3.5H^{13}NMR Predictions for Copper complex

NMR values confirmed the metal C-H shifts for quinolone component in neocuproine ligand as follows.
Node Shift Base + Inc. Comment (ppm rel. to TMS)
Node CH at shift 9.14 ; CH- 6.99 ; 7.26 quinoline ; methy- 2.77 1.69 1 alpha -
1:C*N*C*C*C*C*1 CH 8.16 : 8.00 , quinolinuinoline CH CH 8.16 : 8.00 quinoline

CH 7.89 , 7.68 quinoline , CH 7.89, 7.43 quinoline
CH3 -2.53 0.86 methyl, 1.69 1 alpha -1:C*N*C*C*C*C*1,
CH3 1.89 0.86 - methyl
Coupling constants as showed in the following figure predicted the position and skeleton of the complex – Ligand structure.

^{1}H NMR Coupling Constant Prediction

shift	atom index	coupling partner,	constant and vector
9.14	9		
	14	7.5	H-C*C-H
	13	1.5	H-C*CH*C-H
6.99	18		
	17	7.5	H-C*C-H
6.99	29		
	30	7.5	H-C*C-H
6.83	3		
	2	7.5	H-C*C-H
7.40	13		
	14	7.5	H-C*C-H
	9	1.5	H-C*CH*C-H
8.16	17		
	18	7.5	H-C*C-H

3.5 Mode of action of phytocompounds in Redox probe

The plant extracts consisted of the compounds as coumarins, quinine, anthraquinone, Phenoliccompounds were identified. They undergo redox reaction in SHT mode with royal blue coloured copper (II) complex then changed the colour as Green Cu (I) as reduced state.This redox potential current was recorded in the electro voltammograms asshown in figures. Height of the peak is proportional to the concentration of redox active species in solution. Voltammetric signals were recorded at room temperature with scan rate of 30mV/s. Antioxidants neutralize free radicals by donating one of their own H/ electron, ending the electron "stealing" reaction. The antioxidant nutrients themselves don't become free radicals by donating an electron because they are stable in either form. Mode of SHT was showed in the figure 3.6.

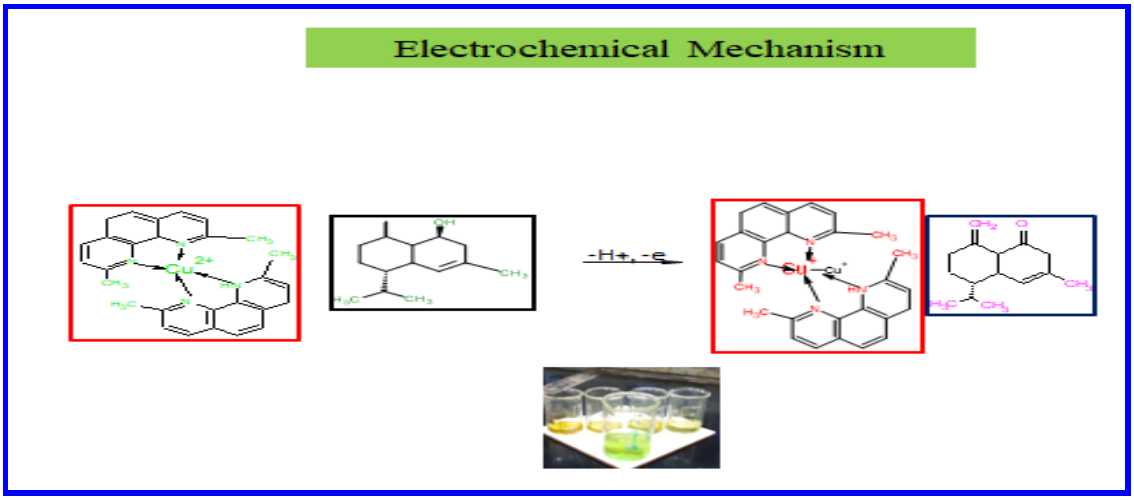


Figure-3.6 SHT mode mechanism of redox probe with phyto compounds of *Coriandrumsativum*

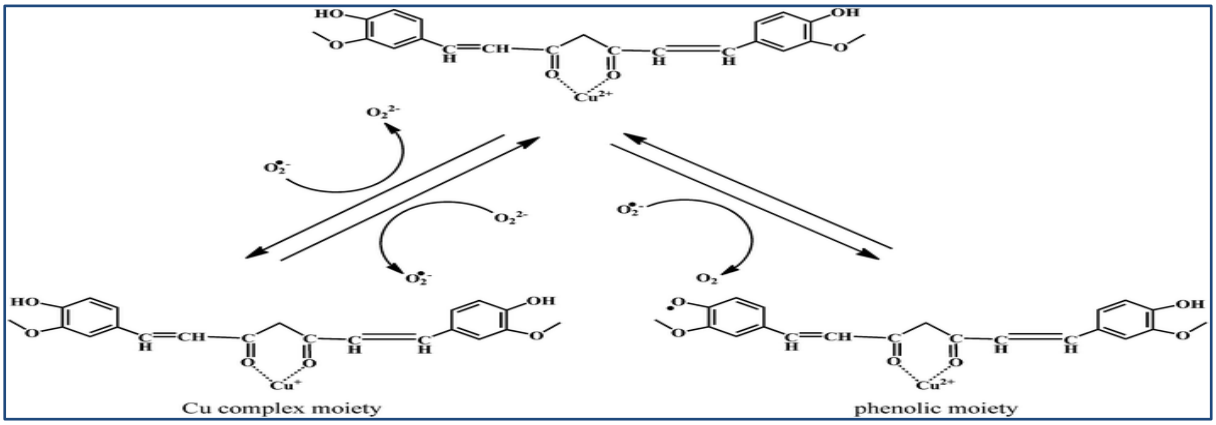
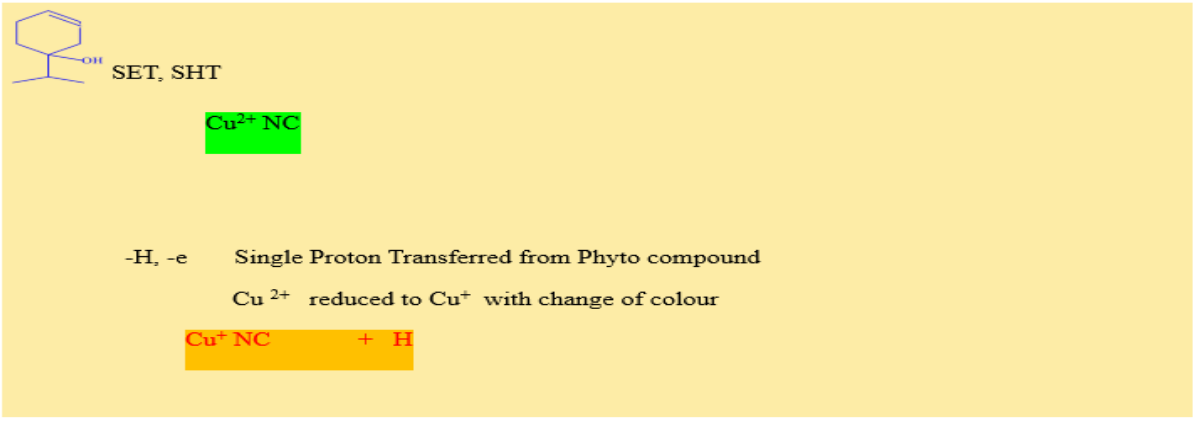


Figure-3.7 SHT mode mechanism of redox probe with phyto compounds of C*urcumalonga*



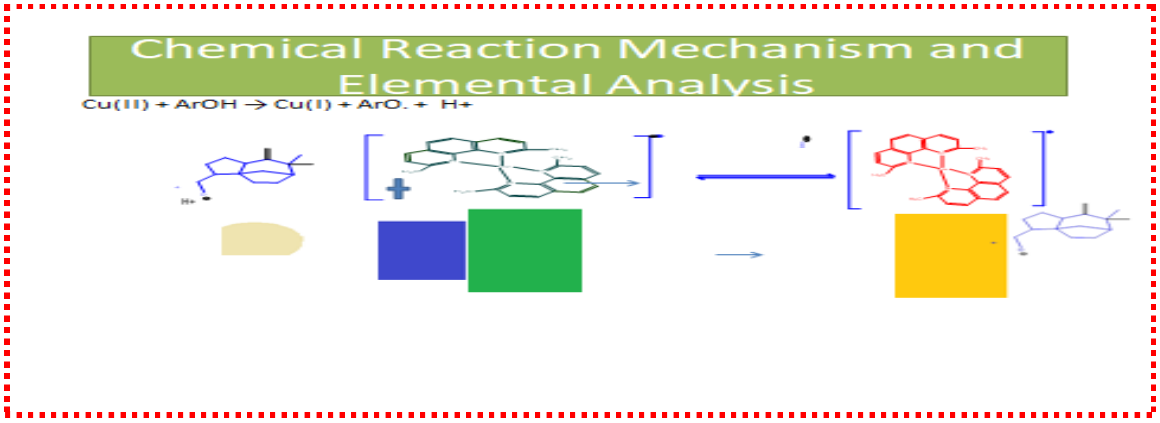


Figure-3.8 SHT mode mechanism of redox probe with phyto compounds of *Vetriver*
Change of colour during each addition of phytocompounds were showed in the figure 3.8.

3.6 Cupric ion reducing antioxidant potential in electrochemical workstation

For the determination of antioxidant ability of phyto compounds, the stock solution of Cu II neocuproine was diluted to 1: 100 mL ratio of water. From this 25 ml was taken as the redox probe and subjected to triple electrode system in the electrochemical work station. Plant extracts in 100, 200, 300, 400,500$\mu\ell$ were also and taken in the micro pipette as Analytes and dropped into the redox probe for all the analysed plant extracts. The height and breadth of the peak showed clearly only at 500 $\mu\ell$ of each sample.

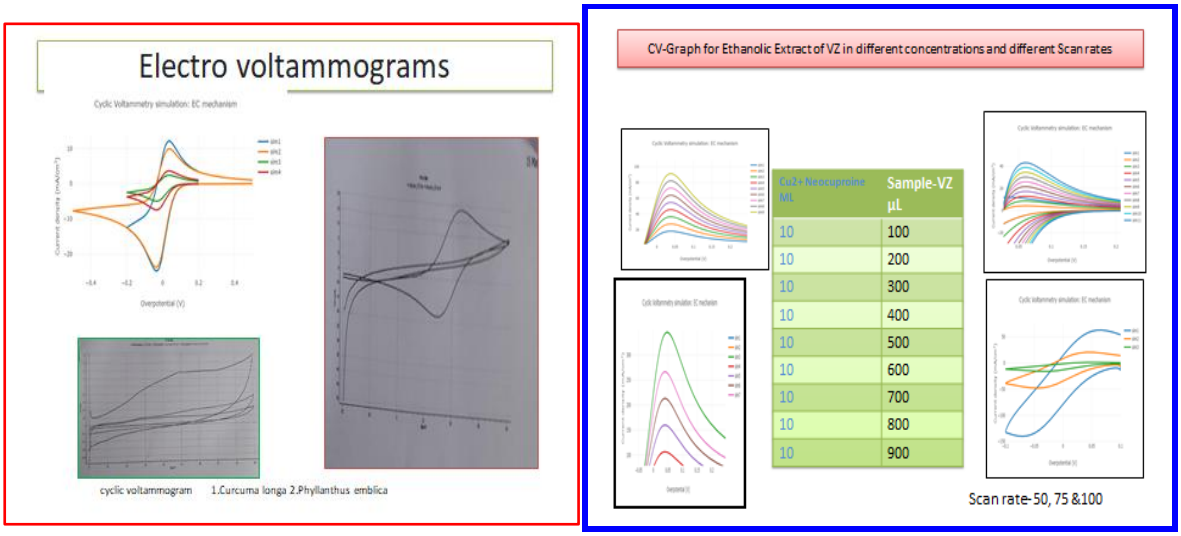


Figure -3.9Showed the anodic and cathodicpeaks with different scan rates for C*urcumalonga*

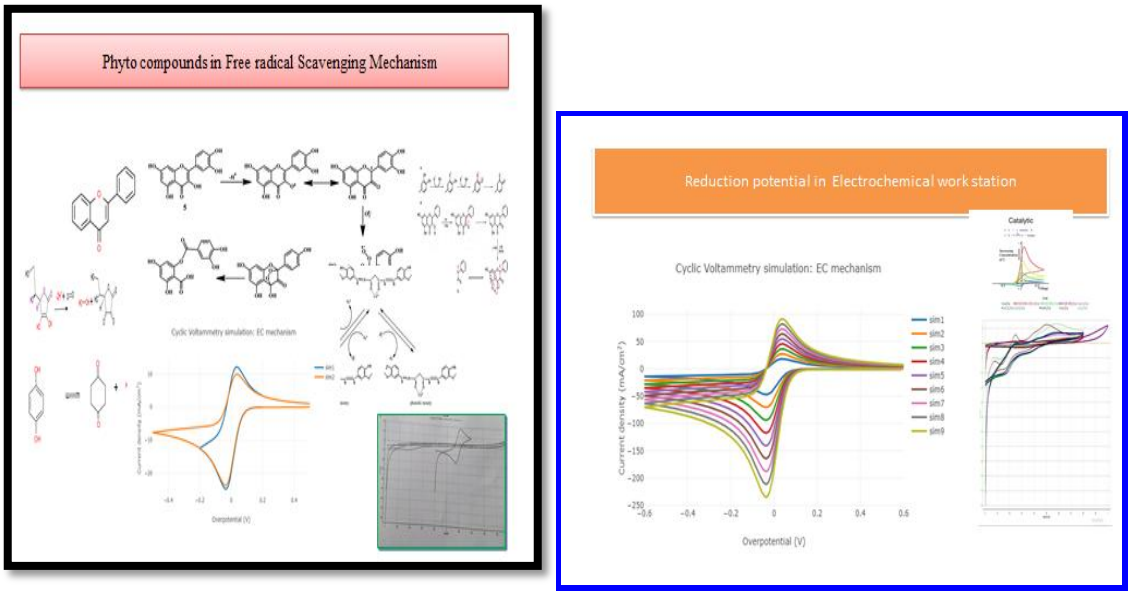


Figure -3.10 Showed the anodic and cathodicpeaks with different scan rates for *, Coriandrumsativum and Piper nigrum*

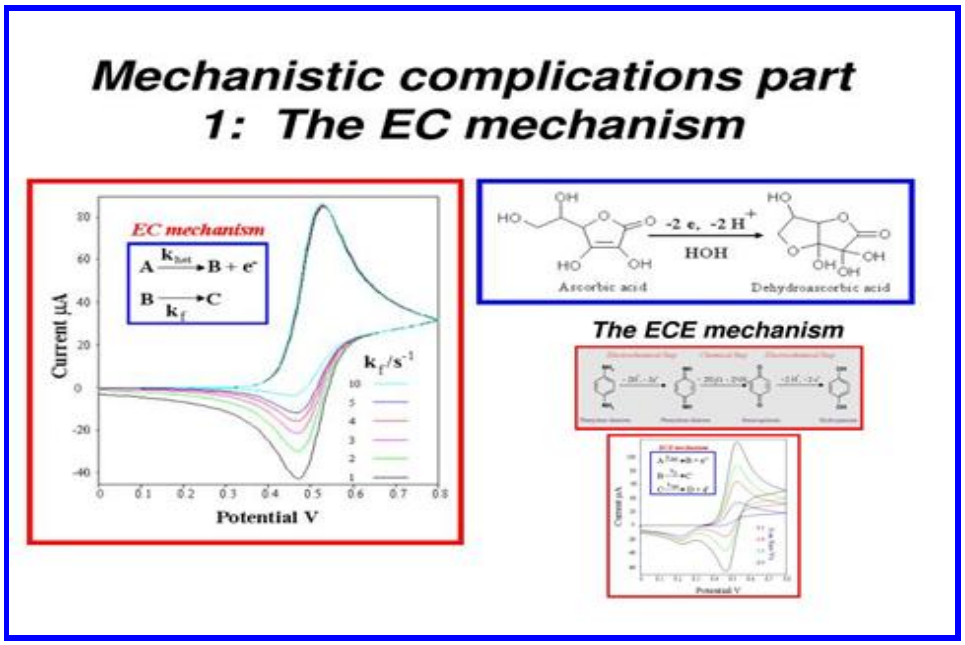


Figure -3.11Showed the anodic and cathodic peaks for *Phyllanthus emblica.*

As many unknown structures could occur in the extract, since current is an additive magnitude, Ipa is an indication for this concentration The surface area (Q) under the oxidation peak in a CV represented charge Q via the linear relation between applied voltage, V, and time, t: the scan rate. Q is more accurate than Ipa, since it measured the total amount of exchanged charge expressed in Coulombs, which can be caused by multiple electro active components in a solution, lower E1/2 and a higher Q are supposed to increase the antioxidant nature of the solution and hight of the current peak explained the high quantity of OH groups involved in redox reaction of that sample The current showed the contribution of all complex structures included in the extract. Figures-3.9 to 3.11 showed the anodic and cathodic peak potential for all the plant extracts. Antioxidant activity can be established by the half-wave potential (E1/2) of the oxidation peak, the potential at half the anodic peak current (Ipa). Antioxidants with low E1/2are stronger electron-donating specie. Low oxidation potentials in samples show their high antioxidant capacity. The results were shown in the table -3.1.

Table-3.1 ΔE values for analysed compounds

Plant Extract	**Copper complex mL**	**Volume of plant Extract µℓ**	**ΔE**
Curcuma longa	10	500	0.367
Phyllanthus emblica	10	500	0.392
Coriandrumsativum	10	500	0.483
Piper nigrum	10	500	0.492
Vetiveriazizanioides root	10	500	0.532

The above mentioned phyto compounds acted as antioxidants and reduced the Cu –II to Cu I by SHT mode and considered as they neutralized the free radicals by giving up some of their own electrons/ Protons hence "off" switch for the free radicals. The release of H/e – were measured in electrochemical work station and identified the (ΔE) reduction potential of the phytochemical extracts as *Curcuma longa*-0.337mV, *Phyllanthus emblica*-0.392MV , *Coriandrumsativum*- 0.483, Piper nigrum- 0.492 , *Vetiveriazizanioides* root- 0.532 for each 500µℓof plant extract in 25ml of Copper complex. Lower the reduction potential value showed higher the antioxidant capacity. Among these plant extracts *Curcuma longa* at 0.337V and *Phyllanthus emblica* at 0.392V were observed as the best anti-oxidants.This may be due to large number of phenolic compounds and ascorbic acids found in the above two compounds when compare o other plants.

IV. Conclusion

The aim of the project is achieved successfully. Phytochemical compounds were analysed qualitativelyfor *Curcuma longa*,*Phyllanthus emblica*,*Coriandrumsativum*, *Piper nigrum*, *Vetiveriazizanioides* root by sonication. Organic extracts showed larger number of compound than aqueousextracts.Redox SET/ SHT

mode of mechanism were identified in the phyto compounds when they reacted with Copper II complex. Royal blue colour Cu-II complex was reduced by this mode of phyto compounds and formed green Cu- I complex. The redox mode was quantified as peak current and reduction potential values by Electrochemical work station.Lower the reduction potential value showed higher the antioxidant capacity. Among these plant extracts *Curcuma longa* at 0.337V and *Phyllanthus emblica* at 0.392V were detected as the best anti-oxidants.This may be due to large number of phenolic compounds and ascorbic acids found in the above two compounds when compare o other plants.Overall results of study suggested that all plants contained one or other pharmacologically active constituent in them and take necessary steps to identify their sensor activity in corrosion in near future.

Acknowledgement

This work was financially supported by Managing Board of V.V.Vanniaperumal College for Women, Virudhunagar and instrumental supported by Department of Biotechnology, New Delhi under DBT-Star College Scheme**.**OurSincere thanks to "The Managing Board and Principal of V.V.Vanniaperumal College for Women Virudhunagar for their constant support and encouragement and also to Prof.V.S.Vasantha, Head of the Department of Natural Products Chemistry, School of Chemistry, Madurai Kamaraj University Madurai for providing electrochemical workstation to carry out this work.

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M.Amutha. "Redox Mode of SET/SHT in the determination of Antioxidant potential of Phyto Chemical compounds / Cu (II)Nc complex in Electrochemical work Station." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 15(07), (2022): pp 15-23.