Antioxidant Activity Evaluation of Hygrophyila Auriculata and Azima Tetracantha Extracts by An Electrochemical Method

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Abstract: The antioxidant activity potential of Hygrophila auriculata and Azima tetracantha extracts were investigated against superoxide anion radical while employing cyclic voltammetry technique. The voltammetric response of the electrochemically generated superoxide anion radical in DMSO was monitored in the absence and presence of the plant extracts. The decrease in the current was interpreted in terms of antiradical activity of the added extracts. The thermodynamic feasibility of the radical scavenging by extracts were accounted in terms of antioxidant activity coefficient (Kao), binding constant (Kb) and standard Gibbs free energy (-ΔGo). Cyclic voltammetry is expected to be a simple method for screening antioxidants and estimating the antioxidant activity of foods and medicinal plants.

Keywords: Antioxidant activity, superoxide anion radical, cyclic voltammetry, binding constant, Hygrophila auriculata, Azima tetracantha.

I. INTRODUCTION

Free radical in biological system is ubiquitous and they can come from a variety of external sources. Free radicals are either formed by cells through various metabolic processes or produced due to the exposure to different types of radiations, chemicals, environmental stress and the sun1. Oxygen is an essential ingredient of the cell metabolism and is capable of producing reactive oxygen species (ROS), most of which are free radicals; such as superoxide ($O2.^{-}$), hydroxyl ($OH\cdot$), hydrogen peroxide (H_2O_2), alkyl (R.), alkoxyl (RO.) and peroxyl (Roo.) radicals. ROS are responsible for several degenerative processes and diseases such as aging, emphysema, inflammation, certain cancers, atherosclerosis, liver injury and many others2. Because of restrictions on synthetic antioxidants due to their carcinogenicity, interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicine materials to replace the synthetic ones+.

Hygrophila auriculata (Schum) Heine (Syn) Asteracantha longifolia Nees, Ascanthaceae, is a wild herb commonly found in moist places on the banks of tanks ditches and paddy fields throughout India and is one of the Ayurvedic drug "Kokilaksha". The plant has shown to possess hypoglycemic activity4, hepatoprotective activity5, antitumour 6activity. According to Daniel, 2005 flavonoid apigenin with derivatives apigenin -7-O-glucuronide and 7-O- glucoside) occured in leaves and flowers while Balraj et al., 1982 reported another flavonoid luteolin (and derivative luteolin-7-rutinoside) from leaves. Azima tetracantha Lam.(Salvadoraceae) commonly known as "mullchangu" is a glabrous, rigid, rambling, thorny shrub.. The ethno botanical survey reveals the usage of this plant as an unique folk medicine by the adivasis (tribal)7. It is a powerful diuretic given in rheumatism, dropsy, and chronic diarrhea and as a stimulaht tonic after confinement8. The leaves are found to contain azimine, azcarpine, carpine and isorhamnitine -3-O-rutinoside etc 9.

Despite the significant documented literature as mentioned above, there is rarely a scientific report regarding their use as free radical scavenger i.e as an antioxidant. To the best of our knowledge no electrochemical studies has been made so far on the subject matter. Keeping in view the aforementioned points the present work was aimed to evaluate, for the first time, the antioxidant activity of H. auriculata and A. tetracantha leaf extracts.

II. MATERIALS AND METHODS

Instrumentation and Reagents

Cyclic voltammetric measurements were carried out using CHI650C (Utrecht, The Netherlands) along with the software GPES 4.9. All the experimentation was made in a double walled electrochemical cell (Model K-64 PARC) and conventional three electrode system such as Glassy Carbon (GC) electrode having area 0.034 cm2, Platinum wire as a counter electrode and silver-silver chloride (Ag/AgCl, 3M KCl) as a reference electrode were employed. Dimethyl sulphoxide (DMSO) of analytical grade was purchased from LAB-SCAN Analytical sciences. Tetrabutyl ammonium perchlorate (TBAP) of electrochemical grade (99%) from Fluka company used as supporting electrolyte and its concentration was kept 0.1M.

Plant sources and Identification

Fresh and disease free leaf of H. auriculata and A. tetracantha were collected from local village Korampallam of Thoothukudi district. The plant was identified with the help of local flora and voucher specimen, preserved in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu, India.

Qualitative Phytochemical Analysis

Phytochemical screening was carried out as per the procedure10.

Estimation of Total phenolic and flavonoid content

Total phenolic and flavonoid content were estimated as per the standard procedure 11, 12.

Extraction of herbs

The H. auriculata and A. tetracantha were shade dried separately at room temperature and the dried leaf and bark were powdered in a wiley mill. Then 5g of each was soaked separately in 50ml of DMSO. After one weak the extract was purified by repeated filtration. The H. auriculata and A. tetracantha extracts were used as such for cyclic voltammetric measurements. The calculated percentage yields (w/w) of H. auriculata and A. tetracantha extracts were 11.4% and 12.6% respectively.

Procedure

Superoxide anion radical was generated in DMSO containing 0.1M TBAP. The scan rate was kept 50 mV/s and potential window -0.8V to + 1.4V. The additions of an extract were made volumetrically as the concentration of the compounds of extracts was not possible to be calculated. The extract was added incrementally to the in situ generated radical and resultant behaviour was recorded. From the change in the shape of the voltammograms the antioxidant activity was assessed qualitatively and quantitated using pertinent mathematical formulations.

III. RESULTS AND DISCUSSION

Phytochemical screening of H. auriculata and A. tetracantha bark extracts

Phytochemical analysis showed the presence of alkaloids, flavonoids, saponins, quinine, phenol and glycosides.

Total Phenolic and Flavonoid Content

The total phenolic content of ethanol extracts of H. auriculata and A. tetracantha were found to be 0.35 g 100g-1 and 0.65g 100g-1 respectively. The total flavonoid content of ethanol extracts of H. auriculata and A. tetracantha were found to be 0.23g 100g-1 and 0.71g 100g-1 respectively. The results revealed that total phenolic content and flavonoid content of ethanol extract of A. tetracantha are higher than the ethanol extract of H. auriculata.

Electrochemical generation of superoxide anion radical

The superoxide anion radical was generated by one electron reduction of the atmospheric molecular oxygen(O2) dissolved in DMSO at room temperature(30ºc)and the resultant CV response is presented in (fig.1), having well developed and clear oxidation and reduction peaks with peak separation (ΔEP) value of 60mV.

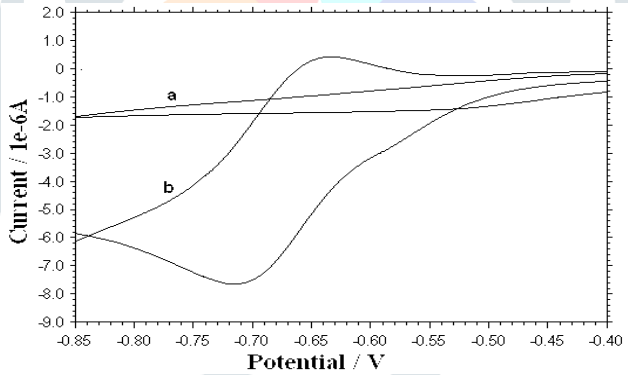

Fig. 1.Cyclic Voltammogram of: (a) medium (DMSO+ TBAP) (b)O2•- in DMSO+ 0.1M TBAP, on GC as working electrode vs. Ag/AgCl as reference at 30ºC with scan rate of 50mV/s.

Effect of extracts on the superoxide radical

The effect of increasing H. auriculata and A. tetracantha extracts concentration on the peak current and peak potential of the superoxide anion radical were investigated. Radical scavenging behavior of H. auriculata and A. tetracantha extracts are checked from minimum concentration upto maximum concentration, 500µL for H. auriculata and 1000µL for A. tetracantha extract by stepwise addition of extracts to the solution having superoxide anion radical (fig 2).

For H. auriculata and A. tetracantha extracts, the maximum scavenging effect i.e., antioxidant activity are observed at 150µLand 500µL which remained constant afterwards.. For such a case the increment was further reduced (Table 1).

The cyclic voltammograms of the superoxide anion radical in the presence of H. auriculata and A. tetracantha extracts showed that the addition of the extract caused proportional decrease in anodic current while the effect on the cathodic current appears to be negligible, which due to the scavenging activity of the added extract 13. The decrease in the current is directly proportional to the concentration of the radical and is a direct measure of the antioxidant activity14.

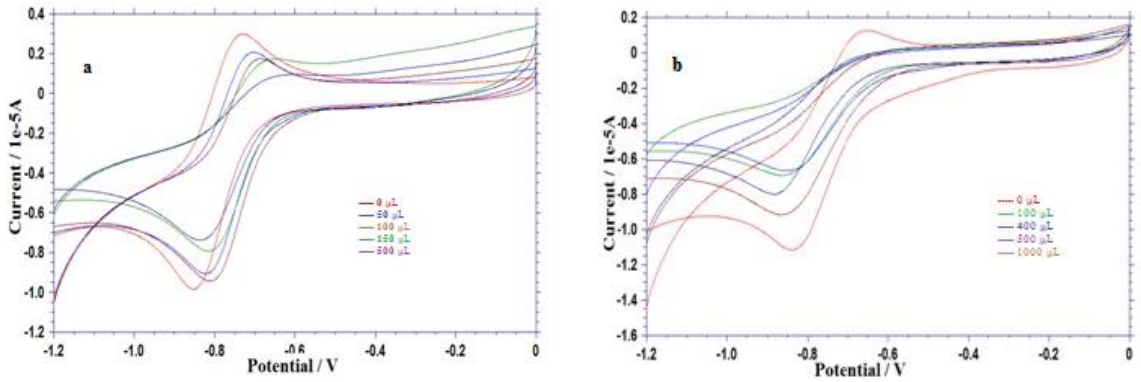

Fig. 2. Cyclic voltammogram of O2•- in the presence of different volumes of H. auriculata (a) and A. tetracantha (b) extracts in DMSO + 0.1 M TBAP on GC as working electrode vs. Ag/AgCl at 30°C with scan rate of 50mV/sec

Table1. Anodic peak current (Ipa), binding constant (Kb), change in free energy of reactions (-ΔG0) and antioxidant activity constant (Kao) for H. auriculata and A. tetracantha leaf extracts

Vol. of Extract (µL)		I_{pa} x 10^{-6}(A)		$K_b x10^{-2}(L^{-1})$		$-\Delta G^0$(kJ/mol)		$K_{ao} x10^{-2}(L^{-1})$	
H.auriculata	*A.tetrcantha*	*H.auriculata*	*A.tetrcantha*	*H.auriculata*	*A.tetrcantha*	*H.auriculata*	*A.tetrcantha*	*H.auriculata*	*A.tetrcantha*
0	0	3.394	1.201	-	-	-	-	-	-
50	100	3.301	-1.648	562	595	35.21	36.74	5.09	36.01
100	400	3.012	-2.376	1268	1402	44.28	49.49	10.458	61.28
150	500	2.503	-3.912	2373	2515	48.52	52.78	16.26	70.06
500	1000	0.450	-8.960	3112	3218	51.74	60.88	16.12	69.96

Thermodynamic parameters

To quantify the results, the strength of interaction between superoxide anion radical and the probable antioxidant in the extract, was estimated in terms of binding constant Kb. Based on the decrease in peak current of superoxide anion radical with increasing concentration of added extracts, the binding constant (Kb) was calculated using following equation 15.

$$\log\left(\frac{1}{[AO]}\right) = \log K_b + \log\left(\frac{I_p}{I_{po} - I_p}\right) \quad (1)$$

Where, Ipo and Ip are the peak currents of superoxide anion radical in the absence and presence of additives, respectively. [AO] is the concentration of the antioxidant. As [AO] is not known, therefore, this term was replaced by the volume of the extracts (ΔVext).

The obtained Kb values of the extracts are higher than the synthetic flavonoids used in similar study16, which confirm the strong affiliation of the compounds present in extracts with the radical and indicate the high probability of the presence of more than one compound in the extract. Another thermodynamic parameter, standard Gibbs free energy, (ΔGo) was calculated using the measured Kb. Sufficiently large negative value of the change in free energy (ΔGo), indicates not only the spontaneity of the reaction between superoxide radical and extracts but it also contributes to the relative stability of the newly formed species, which in turn is a strong evidence of the effectiveness of the extracts for the consumption of free radical.

Antioxidant Activity (AOA)

The relative capacity of polyphenols to scavenge the target radical is determined as antioxidant activity coefficient (Kao). The constant Kao is defined as the ratio of current density values, with and without the addition of substrate to the free radical. To quantity the effect, equation (2)17 was employed with little modification.

$$K_{ao} = \frac{\Delta j}{(j_o - j_{res})\Delta C} \quad (2)$$

Where, Δj- change in the oxygen anodic current density with the addition of the substrate..j_o- limiting current density of oxygen without antioxidant in the solution. jres- residual current density without oxygen in the solution under constant potential. ΔC- change in the concentration of the substrates in mol/L.

The result shows that, the observed antioxidant activity of A. tetracantha leaf extract is higher than the H. auriculata leaf extract due to the presence of high phenolic and flavonoid content in A. tetracantha leaf extract when it is compared to H. auriculata . Kao values confirm the presence of polyphenols or antioxidants as main components of the extracts against superoxide radical.

IV. CONCLUSION

The current work was aimed to investigate the antioxidant character of H. auriculata and A. tetracantha extracts against superoxide anion radical while employing cyclic voltammetric method. The H. auriculata and A. tetracantha extracts cause a decrease in the anodic current of superoxide anion radical which evidently demonstrates their potential antioxidant activity. The results reveal that the existence of potential antioxidant compounds in the extracts of chosen plants. Further research will be completed to research the isolation and identification of main phenolic constituents of the extracts.

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