INTRODUCTION

Phenolic compounds are the major source of antioxidant due to their reducing ability. As we have discussed in the previous chapters, different spectroscopic methods have been used to evaluate the antioxidant activity of natural products which include pure compounds, plant extracts and beverages[1,2]. However, all these procedures suffer from certain limitations of their own, since most of these require the use of specific reagents and tedious and time consuming sample preparation. In recent years, electrochemical approaches for studying redox processes including the antioxidant behavior of phenolic compounds are receiving special importance. Electrochemical methods allow the recording an antioxidant action from the initial stage of the same and it is helpful in recording electron transfer process. As in phenolics, electron transfer is involved, so electrochemical methods are useful for study of such process in phenolic systems. Among the electrochemical methods, Cyclic Voltammetry (CV) is the most useful method for determination of oxidation/reduction potential. In electrochemical method, the oxidation potential of an analyte, the number of electron transferred, rate of the electrode reaction can be determined. The oxidation potential measured by CV has been used to compare the antioxidant strength of compounds[3-7]. Lower oxidation potentials are associated with a greater strength of a given molecule for the electron donation and thus to act as antioxidant.

CV is based on the analysis of the anodic current wave function, which is a function of the reduction potential of a given compound in the sample or a mixture of components. A cyclic voltammogram provides information describing the integral antioxidant capacity without the specific determination of the contribution of each
individual component. Since, polyphenolic compounds are reducing agents by quenching free radicals through electron donation, thus, evaluation of overall reduction power of a sample by CV would reveal its antioxidant activity. CV has been successfully applied to analyze antioxidants present in wine[8], plant extracts[9], phenoile standards[10] and even human plasma[11].

From literature review, a good correlation was shown between the oxidation potential of various antioxidants and their antioxidant efficiency[12]. Simic A. et al. reported the electrochemical behavior and pro-oxidant and antioxidant activity of natural phenoilcs[13]. Arulpriya P. et al. used CV for the evaluation of antioxidant activity of Samanea saman (Jacq.) Merr.[14]. M. Seruga et al. reported that cyclic voltammetry was used for the determination of antioxidant activity of red wine[15]. For the characterization of the antioxidant activities of red wines, Aguirre et al. reported that electrochemical method is a simple, rapid and excellent technique[16]. Electrochemical investigation of animal tissues, different type of tea, plant extracts, coffee and beverage have also been carried out by various researchers[17-19].

It is generally difficult to make a direct correlation of the structural and electronic factors of the phenolics present in alcoholic beverage and plant extracts with their redox behavior due to the complex structures. But, the change of a well-known redox system in presence of the compounds may be studied to record the effect, exerted by the samples i.e. whether it works as radical scavenger or inhibitor of oxidation reaction. Presence of any antioxidant delays the oxidation process by scavenging the radical which will be reflected in the cyclic voltammogram.
We have used CV using the above principle for investigation of antioxidant activity of the selected alcoholic beverage, ‘Haanj’ samples and the extracts of the plant species used for their FC. The aim of our work was to analyze the changes observed in the redox behaviour of 1,4-diaminobenzene (1,4-DAB) in presence of ‘Haanj’ samples and the plant extracts.

SECTION A

INVESTIGATION ON THE SELECTED ‘HAANJ’ BY CYCLIC VOLTAMMETRY

4.1 ‘HAANJ’ SAMPLES USED FOR THE STUDY

The description, collection places and preparation methods of investigated ‘Haanj’ samples have been described in Chapter 2. The five samples, namely BR-1, BR-2, BR-3, BR-4 and RB, were used to investigate their antioxidant activities by Cyclic Voltammetry.

4.2 CHEMICALS USED FOR CYCLIC VOLTAMMETRIC MEASUREMENTS

1,4-diaminobenzene (1,4-DAB) has been used to study the change of its normal redox cycle in presence of the beverage samples. Tetrabutyl ammonium perchlorate has been prepared as per procedure stated in Chapter 2. N,N-dimethyl formamide (DMF) was used as solvent in the measurement.
4.3 ELECTROCHEMICAL MEASUREMENTS OF ANTIOXIDANT ACTIVITY
BY CYCLIC VOLTAMMETRY

4.3.1 Recording of Cyclic Voltammogram of 1,4-diaminobenzene Alone

The cyclic voltammogram of 1,4-diaminobenzene was recorded by dissolving 4mg of 1,4-diaminobenzene in DMF (3mL) with 8mg of TBAP as supporting electrolyte. Pure nitrogen gas was passed through the solution before recording of each voltammogram.

1,4-DAB has a well-defined redox cycle. Overall electrochemical process taking place is represented in Scheme 4.1, where 1,4-DAB can have benzenoid structure on electrochemical oxidation and reduction reaction due to the formation of a radical cation and a diiminium dication, respectively. The well-defined redox cycles of 1,4-DAB are with $E_{1/2}$ at 208mV and $E_{1/2}$ at 674mV in DMF (Figure 4.1). The first oxidation wave was observed at 264mV and the second oxidation wave was observed at 910mV. The first reversible cycle with $E_{1/2}$ at 208mV was due to formation of a cation radical, this radical in the second cycle with $E_{1/2}$ at 674mV transforms to a diiminium dication.

![Scheme 4.1](image-url)
4.4 CYCLIC VOLTAMMOGRAMS OF 1,4-DAB IN PRESENCE OF ‘HAANJ’ SAMPLES

CV of each ‘Haanj’ sample/ plant extract was recorded in the same range where the redox cycle of 1,4-DAB was observed to determine if the tested samples have any oxidation/reduction peaks in the range. It was observed that no peak was shown by the ‘Haanj’ samples in the range where 1,4-DAB exhibits its redox cycles.

In order to study the effect of the ‘Haanj’ samples, to the above solution of 1,4-DAB, 0.1mL of ‘Haanj’ sample was added and mixed thoroughly. Then the cyclic voltammogram of the resulting solution was recorded as the same procedure. This experiment was done separately with each of the ‘Haanj’ sample to observe their effect on 1,4-diaminobenzene.

Then the cyclic voltammogram of 1,4-DAB alone and that of 1,4-DAB in presence of ‘Haanj’ samples were overlaid. From the overlaid cyclic voltammograms,
it was observed that the presence of beverage samples significantly affected the electrochemical behavior of 1,4-DAB.

The effects of all ‘Haanj’ samples were shown in Figure 4.2-4.6 and the shifts and/or absence of anodic potential of the oxidation waves of 1,4-DAB is summarized in Table 4.1. All ‘Haanj’ samples delayed the first oxidation wave which indicates that the samples delay the oxidation of 1,4-DAB to the radical cation, probably by stabilizing 1,4-DAB. The second oxidation wave was not observed in all cases, indicating the complete inhibition of the oxidation and established the potential radical scavenging effect of the samples. Because once the cationic radical has been formed due to radical scavenging ability of the samples, the radical has become a non-radical and the second oxidation reaction was not possible. In BR-1, the first oxidation wave was shifted to 366mV from 264mV (Figure 4.2). On the other hand, for BR-2, BR-3, BR-4 and RB samples, first oxidation peak were observed at Ep=455, 482, 348 and 790mV respectively and beyond that no oxidation peak was observed.

**Table 4.1**: Value of anodic potential of 1,4-DAB with or without ‘Haanj’ samples

<table>
<thead>
<tr>
<th>Entry</th>
<th>Value of anodic potential of 1,4-DAB</th>
<th>1st peak, Ep (mV)</th>
<th>2nd peak, Ep (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,4-diaminobenzene alone</td>
<td>264</td>
<td>910</td>
</tr>
<tr>
<td></td>
<td>In presence of Haanj samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>‘Haanj’ sample (BR-1)</td>
<td>366</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>‘Haanj’ sample (BR-2)</td>
<td>455</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>‘Haanj’ sample (BR-3)</td>
<td>482</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>‘Haanj’ sample (BR-4)</td>
<td>348</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>‘Haanj’ sample (RB)</td>
<td>790</td>
<td>-</td>
</tr>
</tbody>
</table>
Chapter 4: Investigation of Cyclic Voltammetry

Fig 4.1: Cyclic voltammogram of 1,4-DAB

Fig 4.2-4.6: Overlaid CV of 1,4-DAB alone (shaded) and in presence of ‘Haanj’ samples (not shaded), viz. BR-1, BR-2, BR-3, BR-4 and RB
SECTION B

INVESTIGATION OF THE PLANTS USED IN PREPARATION OF FERMENTATION CAKES BY CYCLIC VOLTAMMETRY

4.5 DESCRIPTION OF PLANT MATERIALS

The plant species used for this study were described in Chapter 2 and the extraction procedure was also described. The concentrated extracts of different solvents were used here for this purpose.

4.6 CYCLIC VOLTAMMOGRAMS OF 1,4-DAB IN PRESENCE OF PLANT EXTRACTS

Before studying the effect of the extracts, CV of each extract was recorded in the range where 1,4-DAB has its redox cycle. It was observed that none of the plant extracts shows any peak or redox cycles in this range.

Then to determine the effects of the plant extracts, on the redox cycle of 1,4-DAB, if any, separate experiments were carried out. For this, at first, the cyclic voltammogram of 1,4-diaminobenzene was recorded as described above and to this solution 4mg of the plant extract was added and mixed thoroughly. Then the cyclic voltammogram of the resulting solution was recorded as the same procedure.

The changes, if any, was studied by overlapping the cyclic voltammogram of 1,4-DAB over the cyclic voltammogram of the same in the presence of the extracts.

Different solvent extracts of plant species used for the preparation of fermentation cakes of ‘Haanj’ showed significant radical scavenging capacity. The results were summarized in Table 4.2 and Figure 4.7 - 4.14.
The cyclic voltammogram of 1,4-DAB in presence of hexane, ethyl acetate and methanol extracts of *F. bhotanica*, showed that first redox cycle was delayed, which indicates that the extracts of *F. bhotanica* delayed the oxidation process of 1,4-DAB to the radical cation probably by stabilizing 1,4-DAB by H-bonding through phenolic –OH groups. The delaying of the oxidation process was in the order: methanol extract > ethyl acetate extract > hexane extract. The second oxidation was not observed in presence of the extracts of *F. bhotanica* (Figure 4.7.1 - 4.7.3). This indicates that these extracts scavenged the cation radical, as soon as it was formed.

In case of hexane, ethyl acetate and methanol extracts of *G. arborescens*, it was also observed that all three extracts delayed the oxidation process of 1,4-DAB and no second oxidation wave was found (Figure 4.8.1 - 4.8.3). On the other hand, for *I. cymosa*, hexane, ethyl acetate and methanol extracts scavenged the cation radical of 1,4-DAB, as a result, it inhibited the second oxidation step and the second wave was absent in their corresponding cyclic voltammograms (Figure 4.9.1 - 4.9.3). The effect of hexane, ethyl acetate and methanol extracts of *L. microphyllum*, *M. malabathricum* and *N. zeylanican* were shown in Figure 4.10.1 - 4.10.3, Figure 4.11.1 - 4.11.3 and Figure 4.12.1 - 4.12.3 respectively. All plant extracts showed effective radical scavenging ability and changed the electrochemical behavior of 1,4-DAB. All extracts delayed the first oxidation process of 1,4-DAB and also shifted to higher potential from 264mV. Similar results were obtained for hexane and ethyl acetate extracts of *R. ellipticus*. However, in presence of methanol extract of *R. ellipticus* no oxidation peak (absence of first and second) was observed which indicates the complete inhibition of the oxidation process of 1,4-DAB (Figure 4.13.3).
Table 4.2: Values of anodic potential of 1,4-DAB with and without plant extracts

<table>
<thead>
<tr>
<th>Entry</th>
<th>Value of anodic potential of 1,4-DAB</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; peak $E_p$ (mV)</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; peak $E_p$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,4-diaminobenzene alone</td>
<td>264</td>
<td>910</td>
</tr>
<tr>
<td></td>
<td><strong>In presence of plant extracts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.1 Hexane extract of <em>F. bhotanica</em></td>
<td>338</td>
<td>-</td>
</tr>
<tr>
<td>2.2</td>
<td>Ethyl acetate extract of <em>F. bhotanica</em></td>
<td>360</td>
<td>-</td>
</tr>
<tr>
<td>2.3</td>
<td>Methanol extract of <em>F. bhotanica</em></td>
<td>386</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>3.1 Hexane extract of <em>G. arborescens</em></td>
<td>322</td>
<td>-</td>
</tr>
<tr>
<td>3.2</td>
<td>Ethyl acetate extract of <em>G. arborescens</em></td>
<td>270</td>
<td>-</td>
</tr>
<tr>
<td>3.3</td>
<td>Methanol extract of <em>G. arborescens</em></td>
<td>354</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>4.1 Hexane extract of <em>I. cymosa</em></td>
<td>380</td>
<td>-</td>
</tr>
<tr>
<td>4.2</td>
<td>Ethyl acetate extract of <em>I. cymosa</em></td>
<td>411</td>
<td>-</td>
</tr>
<tr>
<td>4.3</td>
<td>Methanol extract of <em>I. cymosa</em></td>
<td>600</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>5.1 Hexane extract of <em>L. microphyllum</em></td>
<td>360</td>
<td>-</td>
</tr>
<tr>
<td>5.2</td>
<td>Ethyl acetate extract of <em>L. microphyllum</em></td>
<td>378</td>
<td>-</td>
</tr>
<tr>
<td>5.3</td>
<td>Methanol extract of <em>L. microphyllum</em></td>
<td>440</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>6.1 Hexane extract of <em>M. malabathricum</em></td>
<td>345</td>
<td>-</td>
</tr>
<tr>
<td>6.2</td>
<td>Ethyl acetate extract of <em>M. malabathricum</em></td>
<td>408</td>
<td>-</td>
</tr>
<tr>
<td>6.3</td>
<td>Methanol extract of <em>M. malabathricum</em></td>
<td>366</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>7.1 Hexane extract of <em>N. zeylanica</em></td>
<td>491</td>
<td>-</td>
</tr>
<tr>
<td>7.2</td>
<td>Ethyl acetate extract of <em>N. zeylanica</em></td>
<td>428</td>
<td>-</td>
</tr>
<tr>
<td>7.3</td>
<td>Methanol extract of <em>N. zeylanica</em></td>
<td>433</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>8.1 Hexane extract of <em>R. ellipticus</em></td>
<td>396</td>
<td>-</td>
</tr>
<tr>
<td>8.2</td>
<td>Ethyl acetate extract of <em>R. ellipticus</em></td>
<td>301</td>
<td>-</td>
</tr>
<tr>
<td>8.3</td>
<td>Methanol extract of <em>R. ellipticus</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>9.1 Hexane extract of <em>Selaginella sp.</em></td>
<td>458</td>
<td>-</td>
</tr>
<tr>
<td>9.2</td>
<td>Ethyl acetate extract of <em>Selaginella sp.</em></td>
<td>422</td>
<td>-</td>
</tr>
<tr>
<td>9.3</td>
<td>Methanol extract of <em>Selaginella sp.</em></td>
<td>455</td>
<td>-</td>
</tr>
</tbody>
</table>

It was observed that the cyclic voltammograms of *Selaginella sp.* for hexane, ethyl acetate and methanol extracts were of similar type (Figure 4.14.1 - 4.14.3). First oxidation process was delayed considerably due to inhibition effect of the extracts and after formation of the radical cation, the extracts scavenged it. That is why, the second oxidation peak was not observed.
Chapter 4

Investigation of Cyclic Voltammetry

Figure 4.7.1 to Figure 4.8.3

Overlaid CV of 1,4-DAB alone (shaded) and
Fig. 4.7.1: in presence of hexane extract of F. bhotanica (not shaded).
Fig. 4.7.2: in presence of ethyl acetate extract of F. bhotanica (not shaded).
Fig. 4.7.3: in presence of methanol extract of F. bhotanica (no shaded)

Overlaid CV of 1,4-DAB alone (shaded) and
Fig. 4.8.1: in presence of hexane extract of G. arborescens (not shaded).
Fig. 4.8.2: in presence of ethyl acetate extract of G. arborescens (not shaded)
Fig. 4.8.3: in presence of methanol extract of G. arborescens (no shaded)
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Figure 4.9.1 to Figure 4.10.3

Overlaid CV of 1,4-DAB alone (shaded) and

Fig. 4.9.1: in presence of hexane extract of *I. cymosa* (not shaded).

Fig. 4.9.2: in presence of ethyl acetate extract of *I. cymosa* (not shaded).

Fig. 4.9.3: in presence of methanol extract of *I. cymosa* (no shaded).

Overlaid CV of 1,4-DAB alone (shaded)

4.10.1: in presence of hexane extract of *L. microphyllum* (not shaded).

4.10.2: in presence of ethyl acetate extract of *L. microphyllum* (not shaded).

4.10.3: in presence of methanol extract of *L. microphyllum* (not shaded).
Figure 4.11.1 to Figure 4.12.3

Overlaid CV of 1,4-DAB alone (shaded)
4.11.1: in presence of hexane extract of *M. malabathricum* (not shaded)
4.11.2: in presence of ethyl acetate extract of *M. malabathricum* (not shaded)
4.11.3: in presence of methanol extract of *M. malabathricum* (not shaded)

Overlaid CV of 1,4-DAB alone (shaded)
4.12.1: in presence of hexane extract of *N. zeylanica* (not shaded)
4.12.2: in presence of ethyl acetate extract of *N. zeylanica* (not shaded)
4.12.3: in presence of methanol extract of *N. zeylanica* (not shaded)
Figure 4.13.1 to Figure 4.14.3

Overlaid CV of 1,4-DAB alone (shaded)

4.13.1: in presence of hexane extract of *R. ellipticus* (not shaded)
4.13.2: in presence of ethyl acetate extract of *R. ellipticus* (not shaded)
4.13.3: in presence of methanol extract of *R. ellipticus* (not shaded)

Overlaid CV of 1,4-DAB alone (shaded)

4.14.1: in presence of hexane extract of *Selaginella sp.* (not shaded)
4.14.2: in presence of ethyl acetate extract of *Selaginella sp.* (not shaded)
4.14.3: in presence of methanol extract of *Selaginella sp.* (not shaded)
4.7 CONCLUSION:

Significant effects on the redox cycle of 1,4-diamino benzene were observed for the presence of ‘Haanj’ samples and the different solvent extracts of eight plant species used for preparation of fermentation cakes of ‘Haanj’. In general, all the ‘Haanj’ samples and plant extracts were found to be excellent radical scavenger and good inhibitor in the electrochemical oxidation of 1,4-DAB. In all ‘Haanj’ samples and plant extracts, it was observed that the first oxidation wave was delayed which indicates that the samples delay the oxidation process of 1,4-DAB to the radical cation probably by stabilizing 1,4-DAB. The second oxidation wave was absent in all cases, indicating the complete inhibition of the oxidation and it established the potential radical scavenging effect of the samples. Because once the cationic radical has been formed, it has been scavenged by the samples due to their radical scavenging ability. The radical becomes a non-radical and the second oxidation reaction was not possible. The results showed that the ‘Haanj’ samples and plant extracts might have functioned as antioxidant due to their radical scavenging activities. It has been observed that, Cyclic Voltammetry may be used as an efficient instrumental tool for evaluating the antioxidant capacity of beverage and plant extracts.
REFERENCES


