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Research Article

Antioxidant and Antibacterial Properties of some Traditional Medicinal Plants of North East India

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Abstract

Antioxidant and antibacterial activities of three traditional medicinal plants of North East India viz., *Pogostemon parviflorus, Houttuynia cordata* and *Portulaca oleracea* were evaluated by Cyclic Voltammetry and Agar well diffusion methods respectively. The antioxidative potentials have been determined by monitoring the change of the oxidation potential in the redox cycle of 1, 4-diaminobenzene in presence of the different extracts of the plants i.e., hexane, ethyl acetate and methanol. 1, 4-Diaminobenzene has two well defined reversible redox cycles with $E_{1/2}$ at 218 mV and $E_{1/2}$ at 535 mV in DMF with the oxidation waves due to formation of a radical cation and a diiminium dication, respectively. In the presence of plant extracts either the oxidation(s) is delayed and/or the radical cation is scavenged as soon as it is formed. It is reflected by the delayed appearance of the oxidation waves and disappearance of the second oxidation wave in the cyclic voltammograms of 1, 4-diaminobenzene in presence of the plant extracts. It was also observed that the methanol extracts possessed antibacterial activity against tested Gram-positive and Gram-negative bacteria.

Keywords: *Electrochemical, Antioxidant, Antibacterial, Cyclic voltammetry, North East India.*

INTRODUCTION

The antioxidant properties of biological samples, foods, extracts, and pure substances are measured by using different methods involving free radicals, but none provide quantitative information on the relative ease of oxidation, an important factor in antioxidant properties. Compounds which are antioxidants by virtue of their ability to act as reductants in solution tend to be easily oxidised at inert electrodes [1].

Electrochemical approaches are of special advantage in studies of the antioxidant properties as they allow the recording of the initial stage of antioxidant action and also the oxidation potential of an analyte, the number of transferred electrons, and the rate of the electrode reaction. Therefore, the electrochemical methods can be used for a rapid test of the antioxidant activity of many compounds, including polyphenols [2].

The antioxidant activity including radical scavenging activity of the medicinal plants was determined by cyclic voltammetry (CV). CV is used as an instrumental tool for the evaluation of total antioxidant capacity of low molecular weight antioxidants (LMWA) including polyphenols of human and animal plasma, other body fluids and animal tissue homogenates [3-8]. CV is also used for the evaluation of the total antioxidant capacity of edible plants [9]. Recently we have used CV for the comparative evaluation of antioxidant activities of some fresh and preserved herbal products of North East India[10]. CV can also be used to monitor the effect of the presence of linear phenol aldehyde condensation oligomers on the redox behaviour of 1,4-diaminobenzene and the oligomers were found to delay the oxidation process by stabilizing the system through H-bonding or by any host guest interaction [11, 12]. Here we report the changes observed in the redox behaviour of 1, 4-diaminobenzene in presence of various extracts of three medicinal plants of North

East India viz., *Pogostemon parviflorus* (*Pp*), *Houttuynia cordata* (*Hc*) and *Portulaca oleracea* (*Po*) and also the antibacterial properties of them. Details of the plants are given in Table-1.

MATERIALS AND METHODS

Plant Materials

The plant materials used for this study have been listed in Table 1. The fresh plant samples were collected from their natural habitats from nearby areas of Dibrugarh University. The freshly cut plants were sorted out and shade dried for few days and then at 60°C in an oven and kept in a desiccators.

Reagents and chemicals

1,4-Diamino benzene and tetrabutyl ammonium bromide (TBAB) were purchased from Sigma Chemicals. Hexane, ethyl acetate, methanol, cyclohexane, and N,N-dimethyl formamide were of AR grade of RANKEM, India. All solvents were purified prior to use according to standard procedure. Tetra butyl ammonium perchlorate (TBAP) was prepared as follows. A saturated solution of 8.4 g of TBAB in 18 ml of H_2O was treated with 2.1 ml of aqueous 70% $HClO_4$. As a result, insoluble perchlorate was formed which was filtered and washed with cold H_2O and dried. Re-crystallization of the TBAP was done in n-pentane – ethyl acetate solution. To a saturated solution of TBAP in ethyl acetate, n-pentane was added to precipitate. Pure TBAP was dried at 100°C under vacuum.

General equipment: Cyclic voltammetry

The cyclic voltammograms were recorded with an Electrochemical Analyzer CH Instrument (Model chi 600c) with three electrodes system comprising of Ag/AgCl reference electrode and two platinum electrodes as working and auxiliary electrodes, respectively.

Procedure: extraction, fractionation and concentration of extracts

100 g of the each of the dried plant material were made into powder form. The dried powder was extracted by a Soxhlet extraction apparatus first with hexane and then with ethyl acetate and methanol, respectively taking about 300 ml of each solvent. The extracts were concentrated to 20 ml at approximately 40°C under reduced pressure in a rotary vacuum evaporator. It is obtained as a concentrated mass.

Electrochemical measurements of antioxidant activity: Cyclic voltammetry

The measurement were done in N,N-dimethyl formamide with TBAP as supporting electrolyte with scan speed 0.1 mV/sec. Pure nitrogen gas was passed through the solution before recording the voltammogram. The EMF values are with reference to ferrocene as standard.

Recording of Cyclic Voltammogram of 1,4-diaminobenzene

The cyclic voltammogram of 1,4-diamino benzene was recorded by dissolving 4 mg of 1,4-diaminobenzene in DMF (3 cm^3) with 8 mg of TBAP as supporting electrolyte.

Recording of Cyclic voltammogram of 1,4-diaminobenzene in presence of the plant extracts

At first the cyclic voltammogram of 1,4-diamino benzene was recorded as described above and to this solution 4 mg of the concentrated extract was added and mixed well. Then the cyclic voltammogram of the resulting solution was recorded as the same procedure. Pure nitrogen gas was passed through the solution before recording of each voltammogram. This experiment was done separately with each of the extracts, prepared to observe their effect on 1,4-diamino benzene.

Antibacterial activity: Agar well diffusion method

Antibacterial activity of methanol extracts of all was tested against five strains of pathogenic bacteria *viz., Enterococcus faecalis, Micrococcus luteus, Proteus mirabilis, Escherichia coli* and *Enterobacter aerogenes* by well diffusion test using 100 µl of suspension containing 10^9 CFU/ml of bacteria spread on plate count agar medium[13]. All bacterial strains have been provided by IMTECH, Chandigarh. A 6 mm well was prepared by puncturing the agar and impregnated with 100 µl of selected plant extracts dissolved in sterile deionised water. Negative control was prepared using deionised water. Tetracycline and streptomycin were used as positive standards to determine the sensitivity in bacterial species tested. The inoculated culture plates were incubated at 37°C for 24 h. The antibacterial activity was determined by measuring the zone of inhibition of growth from the edge of zone to the edge of the well.

RESULTS AND DISCUSSIONS

Electrochemical study:

The effect of plant extracts on the electrochemical behaviour of 1,4-diaminobenzene has been studied with the help of cyclic voltammetry. 1,4-diaminobenzene has been chosen as this is an amine with well-defined redox cycle (Scheme 1) and hence

any change occurred to the redox behaviour may be studied conveniently by cyclic voltammetry. Scheme 1, where 1,4-diamino-benzene can have benzene-benzenoid structure on electrochemical oxidation and reduction reaction. It is an amine having well defined redox cycles with $E_{1/2}$ at 218 mV and $E_{1/2}$ at 535 mV in DMF with the oxidation waves (Figure 1) due to formation of a radical cation and a diiminium dication, respectively (Scheme 1). The first oxidation wave was observed at 230 mV and the second oxidation wave was observed at 620 mV. The first reversible cycle with $E_{1/2}$ at 218 mV is due to formation of a cationic radical, this radical in the second cycle with $E_{1/2}$ at 535 mV transforms to a diimine.

Before monitoring the effects of the presence of different plant extracts on the redox cycles of 1, 4-diaminobenzene, the electrochemical behaviour of the plant extracts have been monitored. The plant extracts under study do not have any redox peaks in the region where 1, 4 – diamino benzene shows its redox cycles. The overall redox reactions of 1, 4-diamino benzene in presence of the plant samples have been significantly affected.

The effects of different extracts of all three plant species are shown in figures 2(a) - 2(i). Most of the samples significantly affected the overall redox reactions of 1, 4- diaminobenzene indicating the profound radical scavenging effect of the extracts. In presence of most of the extracts, the first oxidation wave was delayed which indicates that the plant extracts delay the oxidation process of 1,4-diaminobenzene to the radical cation probably by stabilizing 1,4-diaminobenzene by H-bonding through phenolic –OH groups. In presence of all the three extracts of *P. parviflorus*, the first redox cycle has disappeared indicating the complete inhibition of the oxidation while in case of all other plants, the first oxidation wave was delayed [Figure-2(a)-2(i)].

For the second oxidation wave, it was observed that in presence of all other extracts, it was not at all observed. It indicated that the profound radical scavenging effect of the extracts. Because once the cationic radical has been formed, due to radical scavenging ability of the extracts, the radical has become a non-radical and the second oxidation reaction was not possible.

The scavenging of the cation radical may be analogous to the following mechanism, illustrated in scheme 2. This mechanism is illustrated by taking BHT (Butylated Hydroxy Toluene), a known antioxidant. In this mechanism, BHT can donate a hydrogen atom to any free radical and thus the free radical becomes a non-radical and become unreactive.

Delay of the first oxidation wave may be due to stabilization of 1,4-diamino benzene, such that a higher oxidation potential was required for the first oxidation process. And once the cationic radical has been formed, its behaviour also was changed due to the presence of the plant extracts. In case of the three plant extracts mentioned above, due to the instant scavenging ability of the polyphenolic compounds present in the plant extracts, the radical has become a non-radical and the second oxidation reaction was not possible. For the other plant extracts, the second redox cycle is also delayed. It may be due to stabilization of the cation radical by the polyphenols present in the plant extracts. The inhibition of the first redox cycle and the scavenging of the radical or delaying of its oxidation have been illustrated in the cyclic voltammograms in Figure-2(a) - 2(i) and in Table 2.

Antibacterial Activity

For study of antibacterial activities of the plants, five standard bacterial strains and Agar well diffusion method had been employed. The methanol extracts of three medicinal plants was tested against three Gram-positive bacteria and two Gram-negative bacteria and all were found to have antibacterial activity. The zone of inhibition was tested for the methanol extracts of all the three plants.

As summarized in Table-3, the methanol extracts of three plant species strongly inhibited the growth of one Gram-positive *(Enterococcus faecalis)* and two Gram-negative *(Enterobacter aerogenes* and *Escherichia coli)* bacteria tested. The maximal zones of inhibition range from 6 to 10 mm. The methanol extracts showed no activity against two Gram-positive bacteria viz. *Microccocus luteus* and *Proteus mirabilis*. The highest inhibition was found in *Houttuyinia cordata* and *Portulaca oleracea* against *Enterobacter aerogenes* (10 mm and 9 mm respectively). Since the bacteria used in this study involve in different types of diseases such as *Escherichia coli* can cause severe cramps, diarrhoea and urinary tract infections; *Enterobacter aerogenes* causes bacteremia, lower respiratory tract infections, skin, soft-tissue infections and urinary tract infections and *Enterococcus faecalis* involves in the infections of urinary tract, bacteremia, meningitis, and other infections in humans, and these were significantly inhibited by the plant extracts, so, there is scope of exploiting these plant samples for isolation of the active principles to be used as antibiotic against the above mentioned bacteria.

CONCLUSION

The redox behavior of 1,4-diamino benzene has been altered in presence of the plant extracts. All the samples shifted the redox cycles of 1,4-diamino benzene to higher oxidation potential. All most all samples have delayed the first oxidation wave slightly and the second oxidation wave was not at all observed, indicating that the instant scavenging of the cation radical, as soon as it is formed. It was also observed that the methanol extracts of three plant species strongly inhibited the growth of one Gram-positive and two Gram-negative bacteria tested. Thus the investigated plant samples are potential antioxidants and antibacterial, which may be functional as prophylactic agents while acting as drug.

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Table – 1
Plants used under investigation

Sl. No.	Plant names	Parts use/Form of use	Method/purpose of use
1	Pogostemon parviflorus Benth [Lamiaceae]	Leaf and root	Leaves are taken as medicinal vegetables in cooked form and considered good for liver problems
2	Houttuyinia cordata Thunb. [Saururaceae]	Leaf	Leaves are taken as medicinal vegetables in cooked form and good for liver functions
3	Portulaca oleraceae L. [Portulaceae]	Whole plant	Added to curries to give a sour and cooling taste. It is considered good for dysentery etc

Table-2
E_p values of 1, 4-diaminobenzene alone and in presence of various plants extracts

Sl. No.	Plant extracts	1st Peak E_p in mV	2nd Peak E_p in mV
1	None	230	620
2	Pp (Hex)	-	-
3	Pp (EtOAc)	-	-
4	Pp (MeOH)	-	-
5	Hc (Hex)	285	-
6	Hc(EtOAc)	275	-
7	Hc (MeOH)	332	-
8	Po (Hex)	246	-
9	Po (EtOAc)	252	-
10	Po (MeOH)	254	-

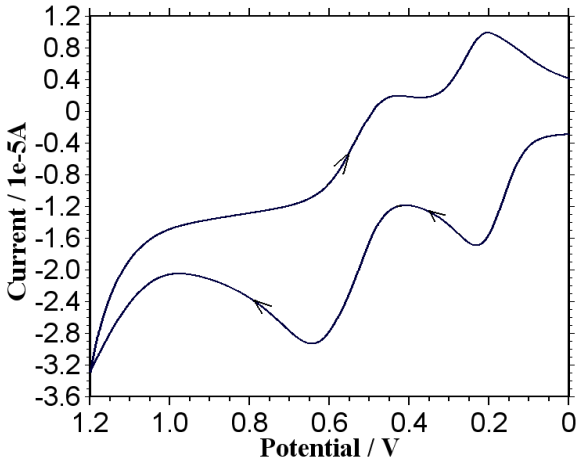
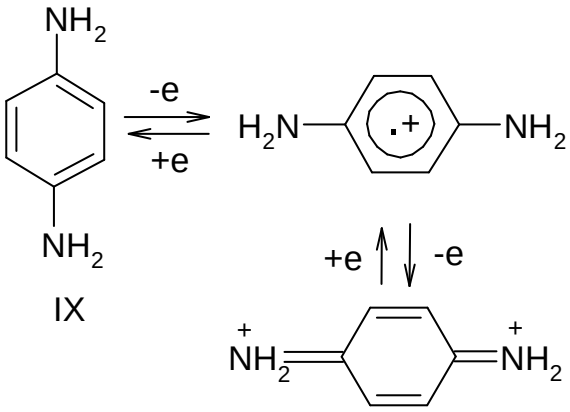
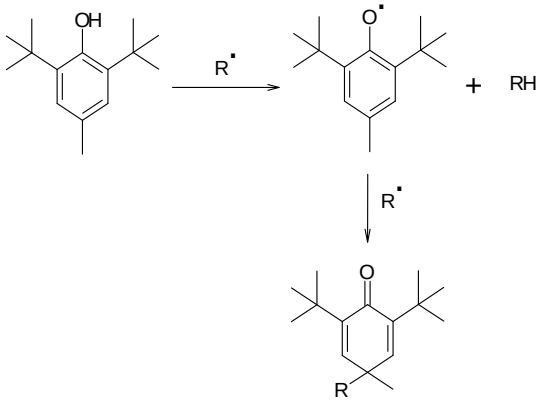


Figure1
Cyclic voltammogram of 1, 4-diaminobenzene



Scheme1



Scheme 2

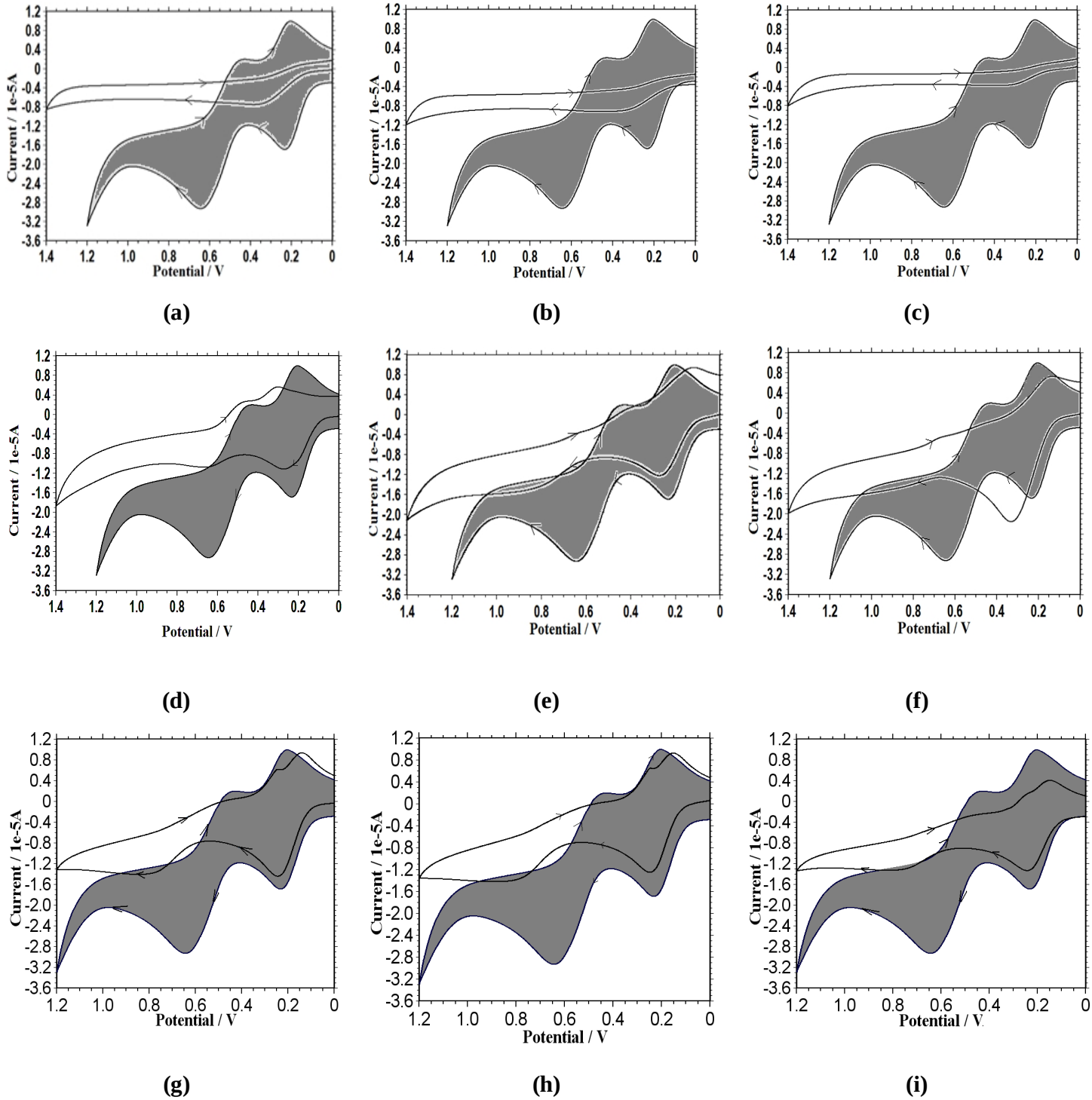


Figure-2

CV of 1,4-diaminobenzene alone (shaded) and that in presence of (a) *Pp* hexane extract, (b) *Pp* ethyl acetate extract, (c) *Pp* methanol extract, (d) *Hc* hexane extract, (e) *Hc* ethyl acetate extract, (f) *Hc* methanol extract, (g) *Po* hexane extract, (h) *Po* ethyl acetate extract, (i) *Po* methanol extract

Table-3
Zone of inhibition of methanol extracts of three medicinal plants

Bacterial species	Zone of inhibition (mm)			
	Antibiotic	*Pogostemon parviflorus*	*Houttuynia cordata*	*Portulaca oleracea*
Enterococcus faecalis (gram positive)	Tetracycline 11	8	6	8
Micrococcus luteus (gram positive)	Tetracycline 14	-	-	-
Proteus mirabilis (gram positive)	Tetracycline 13	-	-	-
Enterobacter aerogenes (gram negative)	Tetracycline 12	8	10	9
Escherichia.coli (gram negative)	Streptomycin 11	7	8	7

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