Indian Journal of Natural Products and Resources
Vol. 3(1), March 2012, pp. 40-47

Comparative evaluation of antioxidant activities of some fresh and preserved herbal products of North East India by cyclic voltammetry

Manas Pratim Boruah, Dhrubajyoti Mahanta and Jyotirekha G Handique*
Department of Chemistry, Dibrugarh University, Dibrugarh-786 004, Assam, India

Received 22 December 2010; Accepted 20 September 2011

Antioxidant activities of fresh and preserved forms of three herbal products, *Bambusa balcooa* Roxb., *Brassica nigra* (Linn.) Koch and *Garcinia morella* Desr., used by the people of North East India have been evaluated by cyclic voltammetry. The antioxidative effects have been evaluated by monitoring the change of the oxidation potential in the redox cycle of 1,4-diaminobenzene in the presence of hexane, ethyl acetate and methanol extracts of the herbal products. 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 herbal 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 the presence of herbal extracts.

Keywords: Antioxidant, *Bambusa balcooa*, *Brassica nigra*, Comparative evaluation, Cyclic voltammetry, Fresh herbal products, *Garcinia morella*, Preserved herbal products.

IPC code; Int. cl. (2011.01) — A61K 36/00, A61P 39/06

Introduction

Many phytonutrients such as carotenoids, tocopherols and polyphenols have attracted attention as these are proved to be able to act as antioxidant[1], protect the structural integrity of cells and tissues[2] and reduce the incidence of chronic diseases, cancer and aging in humans[3]. Consequently, edible plants containing such antioxidant components form an important part of human diet. Plant based foods which are taken in their preserved forms show change in their composition from the fresh form. So, it is necessary to assess the change in their antioxidant properties on preservation. Electrochemical methods have been successfully used for the evaluation of antioxidant capacity of buckwheat products after hydrothermal treatment[4].

Antioxidant activities of biological samples, foods, extracts and pure substances are evaluated by using various techniques based on different mechanisms of antioxidant action[5]. A simple electrochemical method has been developed[6] by using flow-through column electrolysis for estimating the antioxidant activity of flavonoids based on measurement of half-wave potentials.

Although Cyclic voltammetry (CV) is employed to investigate the antioxidant activity comparatively late, studies showed that it is of special advantage in studies of the antioxidant properties of polyphenols[7,8], because in polyphenols electron transfer is involved. The oxidation potential of an analyte, the number of transferred electrons and the rate of the electrode reaction can be determined. CV was also applied to determine the antioxidant properties of wine polyphenols where oxidation potentials were successfully used for comparison of the antioxidant activities of phenolic acids, flavonoids, tocopherols, etc.[9] and had also been used for determination of the total antioxidant capacity of edible plants[10,11]. However, initially, the CV measurements were employed for getting information concerning the integrated antioxidant capacity, which arises from the low molecular weight antioxidants (LMWA), without the specific determination of the contribution of each individual component[12]. But of late, the relative contribution of quercetin and its glucosides to the antioxidant capacity of onion has been determined by CV in combination with spectroscopic methods[13].

*Correspondent author: E-mail: jghandique@rediffmail.com
Phone: +91 373 2370 210; Fax: +91 373 2370323.

In CV method, usually, the reductive potential of a given compound and/or a mixture of compounds is measured by the ability of the compound to donate electron(s), since most of the LMWA are reducing agents, which quench ROS through donation of electron(s) to the ROS, neutralizing its activity. Thus, CV had been used to estimate the reduction potential of extracts of certain herbal formulation and to indicate the presence of oxidisable substrates in the extract[14].

CV can also be used to monitor the effect of the presence of any sample on a well known redox system by recording the change of the system. The effect of some linear phenol-aldehyde condensation oligomers on the redox behaviour of 1,4-diaminobenzene were studied by CV and the oligomers were found to delay the oxidation process by stabilizing the system through H-bonding or by any host-guest interaction[15]. 1,4-Diamino benzene was chosen as it 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. If any plant extract under investigation delay the oxidation processes and/or scavenge the radical, these effects will be reflected in the CV tracing. Here we report the changes observed in the redox behaviour of 1,4-diaminobenzene in presence of various extracts of three herbal products in fresh and preserved forms. The herbal products, viz. *Bambusa balcooa* Roxb., *Brassica nigra* (Linn.) Koch and *Garcinia morella* Desr., have been chosen because of their medicinal and dietary importance.

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 desicator. The preserved forms were prepared according to traditional processes. Before extraction, these have also been dried in shade first and then at 60°C in an oven.

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 400 ml of each solvent. The extracts were concentrated to 20 ml at approximately

Table 1 — Plant materials under investigation

S. No.	Plant names/Family	Parts used/Form of use	Method/purpose of use
1.	*Garcinia morella* Desr./ Clusiaceae	Fresh fruits edible. Sun-dried pericarps of fruits are used as preserved form.	The extract of the preserved form is used in curries to add a sour and cooling taste. Used in dysentery and stomach-ache. Reported to lower high blood pressure.
2.	*Bambusa balcooa* Roxb./ Poaceae	Crushed tender plants. Used fresh as well as in preserved fermented form (called "*kharicha*" in Assamese).	Used as side items with main meal/ added to curries/ in many variations.
3.	*Brassica nigra* (Linn.) Koch. / Brassicaceae	Crushed seeds, in preserved form (called "*kharoli*" in Assamese) are eaten.	Taken as a kind of pickle/side item with main meal.

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.

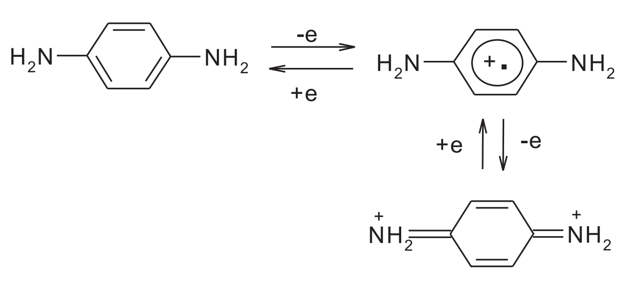
Results and Discussion

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.

Overall electrochemical process taking place is represented in Scheme 1, where 1,4-diaminobenzene 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 (Fig. 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.

The plant samples do not have any redox peaks in the region where 1,4-diaminobenzene shows its redox cycles. The overall redox reactions of 1,4-diaminobenzene in presence of the plant samples have been significantly affected. The effects of different plant extracts are shown in Figs 2-4 and the shifts and/or absence of anodic potential of the oxidation waves of 1,4-diaminobenzene are summarized in Table 2. Among all the plant extracts, hexane and ethyl acetate extracts of fresh *G. morella* and methanol extract of preserved *G. morella* showed mild pro-oxidant activity. Thus, in presence of these extracts, the first oxidation wave of 1,4-diaminobenzene appeared at 201 mV, 210 mV and 200 mV, respectively, rather than at 230 mV (Figs 2a, 2c and 2f). All the other extracts, except the methanol extract of fresh *G. morella,* delayed the first oxidation wave which may be due to stabilization of 1,4-diaminobenzene, such that a higher oxidation potential was required for the first oxidation process.

In presence of the methanol extract of fresh *G. morella*, the first oxidation wave was not at all



Scheme 1

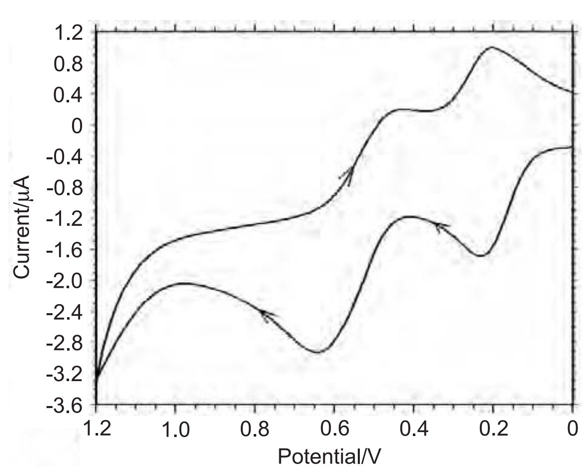


Fig 1—Cyclic voltammogram of 1,4-diaminobenzene

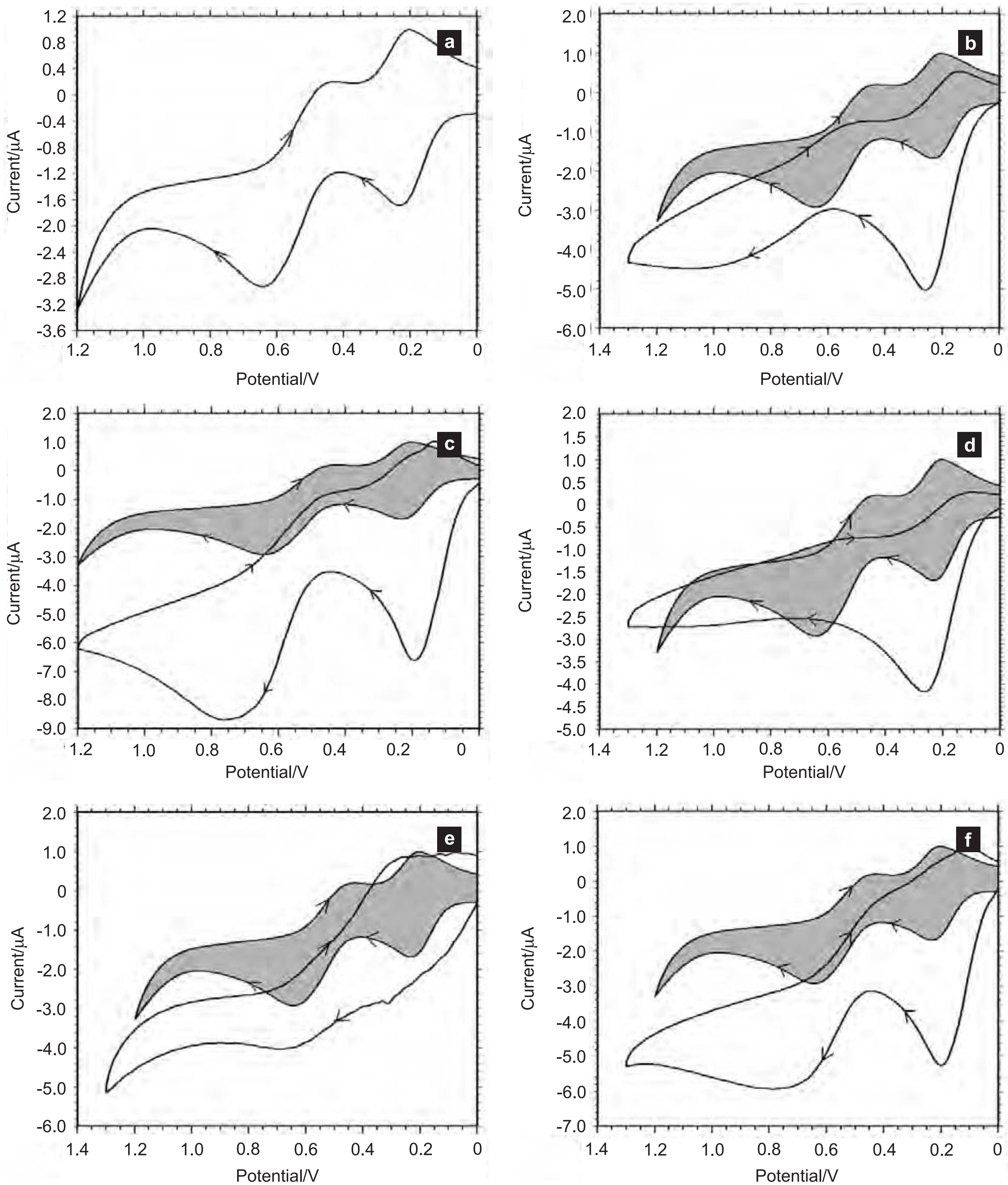


Fig. 2 — CV of 1,4-diaminobenzene alone (shaded) and that in presence of different *Garcinia morella* extracts (not shaded): a-fresh hexane extract, b-preserved hexane extract, c-fresh ethyl acetate extract, d-preserved ethyl acetate extract, e-fresh methanol extract, f-preserved methanol extract

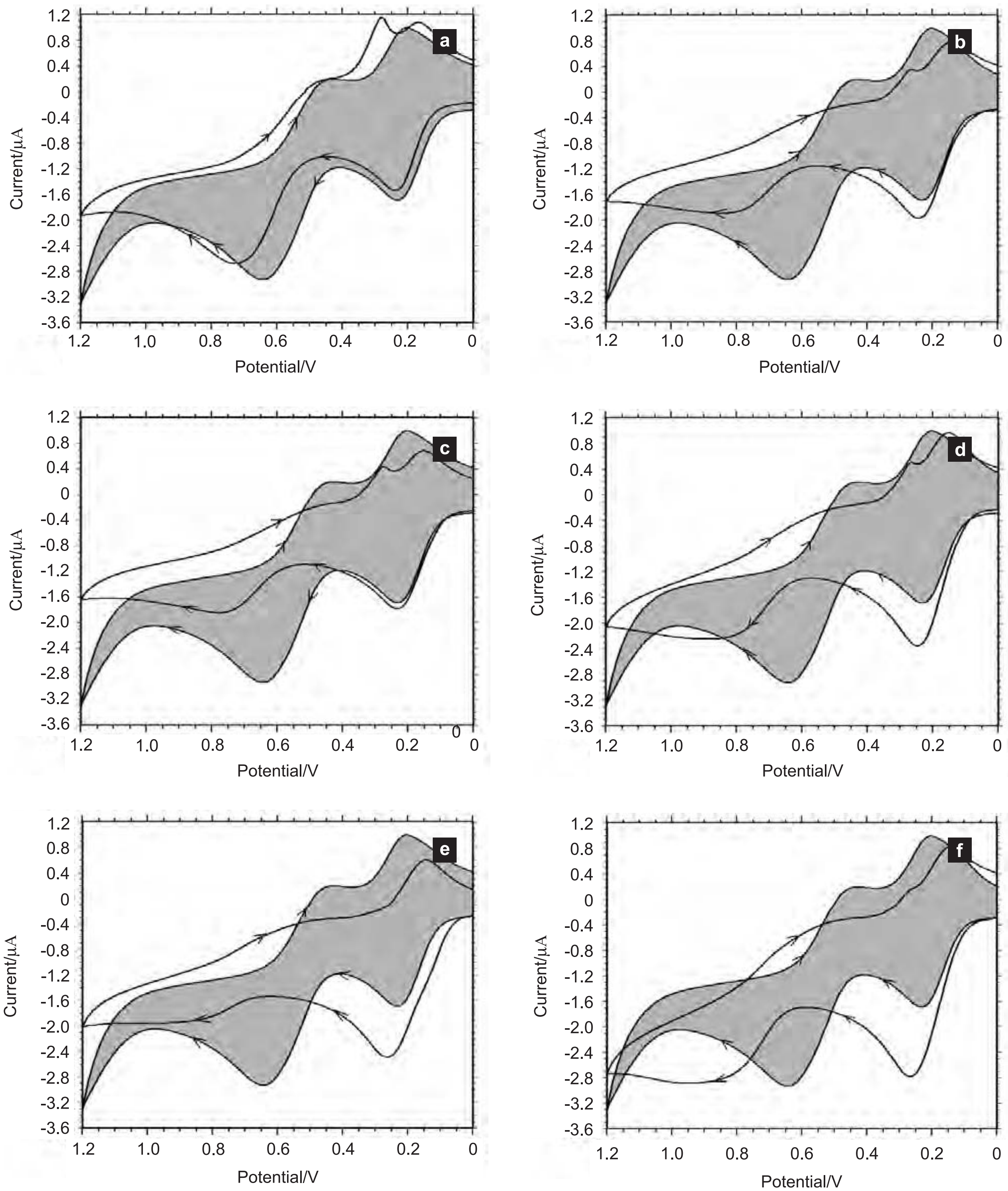


Fig. 3 — CV of 1,4-diaminobenzene alone (shaded) and that in presence of different *Bambusa balcooa* extracts (not shaded): : a-fresh hexane extract, b-preserved hexane extract, c-fresh ethyl acetate extract, d-preserved ethyl acetate extract, e-fresh methanol extract, f-preserved methanol extract

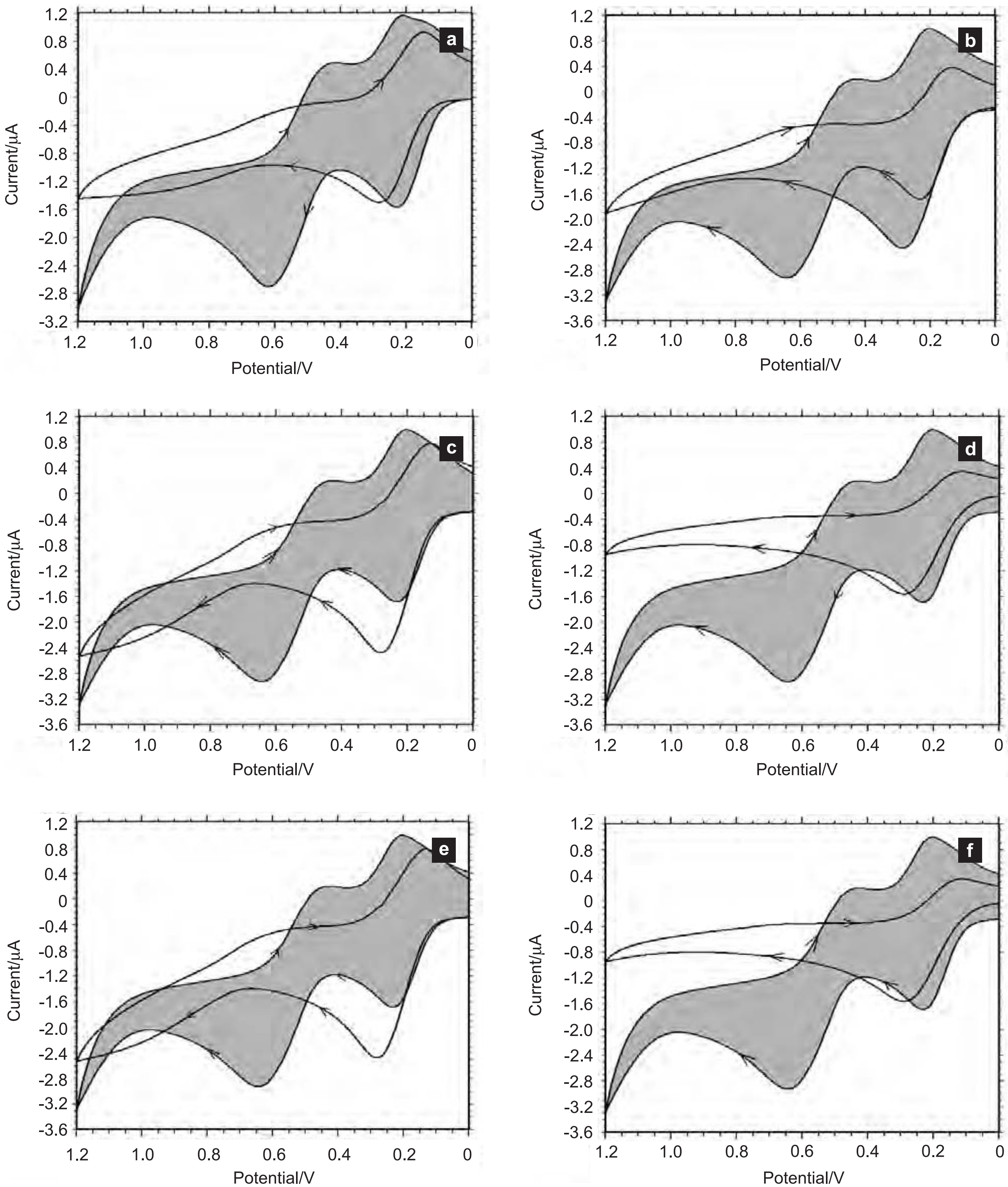


Fig. 4 — CV of 1,4-diaminobenzene alone (shaded) and that in presence of different *Brassica nigra* extracts (not shaded): : a-fresh hexane extract, b-preserved hexane extract, c-fresh ethyl acetate extract, d-preserved ethyl acetate extract, e-fresh methanol extract, f-preserved methanol extract

Table 2 — Values of the anodic potential of 1,4-diaminobenzene alone and in presence of various plant extracts

S. No.	Extract	Values of the anodic potential of 1,4-diaminobenzene			
		1st Peak E_p in mV		2nd Peak E_p in mV	
1	In presence of none	230		620	
In presence of plant extracts		Fresh		Preserved	
		1st Peak E_p in mV	2nd Peak E_p in mV	1st Peak E_p in mV	2nd Peak E_p in mV
2	*Garcinia morella* (Hex)	201	---	264	---
3	*G. morella* (EtOAc)	210	770	247	---
4	*G. morella* (MeOH)	---	---	200	---
5	*Bambusa balcooa* (Hex)	237	717	243	---
6	*B. balcooa* (EtOAc)	234	778	248	---
7	*B. balcooa* (MeOH)	258	---	267	---
8	*Brassica nigra* (Hex)	284	---	289	---
9	*B. nigra* (EtOAc)	283	---	294	---
10	*B. nigra* (MeOH)	276	---	300	---

Hex- Hexane extract, EtOAc-Ethyl acetate extract, MeOH-Methanol extract

observed, indicating the complete inhibition of the oxidation (Fig. 2e). In presence of ethyl acetate extract of fresh *G. morella* and hexane and ethyl acetate extracts of fresh *Bambusa balcooa* (Figs 3a and c) the second oxidation wave is delayed, indicating inhibition of the formation of the diiminium dication.

In presence of all the other extracts except these three, it has been observed that the second oxidation wave was not at all observed, which indicates 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.

Conclusion

The plant samples under study were found to delay the first oxidation wave of 1,4-diaminobenzene and the effect was more profound in the preserved form than the fresh forms. Almost all the samples except the ethyl acetate extract of fresh *Garcinia morella* and the hexane and ethyl acetate extracts of fresh *Bambusa balcooa* showed instant radical scavenging capacity. It may be mentioned that by radical scavenging assays, such as DPPH scavenging assay or ABTS scavenging assay, only the radical scavenging activity of a sample under study can be measured. But by cyclic voltammetry, the inhibition capacity of a sample on an oxidation process can also be measured.

Acknowledgement

Authors are thankful to the University Grants Commission (UGC), New Delhi for financial assistance under SAP-DRS-I. One of the authors (MPB) is thankful to the UGC for a fellowship under the programme.

References

1 Halliwell B and Gutteridge J M C, Free Radicals in Biology & Medicine, 2nd Edn, Oxford University Press, London, UK, 1998.

2 Faure M, Lissi E, Torres R and Videla L A, Antioxidant activities of lignans and flavonoids, *Phytochemistry*, 1990, **12**, 3773-3775.

3 Cody V, Middleton E, Harborne J B and Beretz A, Plant Flavonoids in Biology and Medicine, Vol. 2, Biochemical, Cellular and Medicinal properties, W H Smith, New York, 1988.

4 Zielinska D, Szawara-Nowak D and Zielinksi H, Comparison of spectrophotometric and electrochemical methods for the evaluation of the antioxidant capacity of buckwheat products after hydrothermal treatment, *J Agric Food Chem*, 2007, **55**, 6124-6131.

5 Prior R L and Cao G, *In vivo* total antioxidant capacity: Comparison of different analytical methods, *Free Radic Biol Med*, 1999, **27**, 1173-1181.

6 Yang B, Kotani A, Arai K and Kusu F, Estimation of the antioxidant activities of flavonoids from their antioxidant potentials, *Anal Sci*, 2001, **17**, 599-604.

7 Filipiak M, Electrochemical analysis of polyphenolic compounds, *Anal Sci*, 2001, **17**(Suppl), 1667-1670.

8 Yakovleva K E, Kurzeev S A, Stepanova E V, Fedorova T V, Kuznetsov B A and Koroleva O V, Characterization of plant phenolic compounds by cyclic voltammetry, *Appl Biochem Microbiol*, 2007, **43**, 661-668.

9 Kilmartin P A, Zou H and Waterhouse A L, A cyclic voltammetry method suitable to characterise antioxidant

properties of wine and wine phenolics, *J Agric Food Chem*, 2001, **49**, 1957-1965.

10 Chevion S, Chevion M, Chock P B and Beecher G R, Antioxidant capacity of edible plants: Extraction protocol and direct evaluation by cyclic voltammetry, *J Med Food*, 1999, **2**, 1-10.

11 Campanella L, Martini E, Rita G and Tomassetti M, Antioxidant capacity of dry vegetal extracts checked by voltammetric method, *J Food Agric Environ*, 2006, **4**(1), 135-144.

12 Chevion S, Roberts M A and Chevion M, The use of cyclic voltammetry for the evaluation of antioxidant capacity, *Free Radic Biol Med*, 2000, **28**, 860-870.

13 Zielinska D, Wiczkowski W and Piskula M K, Determination of the relative contribution of quercetin and its glucosides to the antioxidant capacity of onion by cyclic voltammetry and spectrophotometric methods, *J Agric Food Chem*, 2008, **56**, 3524-3531.

14 Naik G H, Priyadarsini K I, Satav J G, Banavalikar M M, Sohani D P, Biyani M K and Mohan H, Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine, *Phytochemistry*, 2003, **63**, 97-104.

15 Handique J G and Baruah J B, Polyphenolic compounds: An overview, *React Funct Polym*, 2002, **52**, 163-188.