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Cyclic voltammetric studies with plant extracts of some traditionally used Indian medicinal plants to evaluate their antioxidant potential

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ABSTRACT

Cyclic voltammetric studies with crude plant extracts of some traditionally used Indian medicinal plants (e.g. *Limnophila indica, Zornia diphyyla, Centipeda minima* and *Rauwolfia tetraphylla*) were carried out in order to evaluate their antioxidant potential in comparison to ascorbic acid (vitamin C).

KEYWORDS

Cyclic voltammetric studies;
Antioxidant potential;
Vitamin C;
Medicinal plant extracts.

INTRODUCTION

Natural products continue to be a major source of biologically active compounds that may serve as commercially significant entities themselves or may provide lead structures for the development of new drugs and thereby valuable medicine. It has been estimated that over 40% of commercially available medicines are originated from natural products[1]. The chemical potential of plants is, however, still virtually unexplored; it is estimated that only 5-15% of the approximately 2,50,000 species of higher plants (terrestrial flora) have been investigated so far chemically and pharmacologically[2,3]. Traditional knowledge associated with biological resources is an intangible component of the resource itself. The utility of traditional knowledge has been realized for its wider applicability as well as its potential of being translated into commercial benefits by providing leads for development of useful products and processes. To a large extent, use of natural products in drug design and development represents the natural evolution of this old tradition. It is now an omni-accepted fact that natural products play a dominant role in the discovery of leads for the development of drugs to treat human diseases. They have traditionally played a major role in drug discovery and still constitute a prolific source of novel chemotypes or pharmacophores for medicinal chemistry. The impact of natural products on the development pipelines of the pharmaceutical industry is unabated. However, continuing improvements in natural products research are needed to continue to be competitive with other drug discovery methods, and also to keep pace with the ongoing changes in drug discovery process. In continuation to our works on plant products of traditionally used Indian medicinal plants, we screened four such medicinal plants, viz. *Limnophila indica* (family: Scrophulariaceae.)[4,5], *Zornia diphylla* (family: Fabaceae)[6], *Centipeda minima* (family: Asteraceae)[4,7,8], and *Rauwolfia tetraphylla* (fruits; family: Apocynaceae)[4,6] in order to assess their anti-

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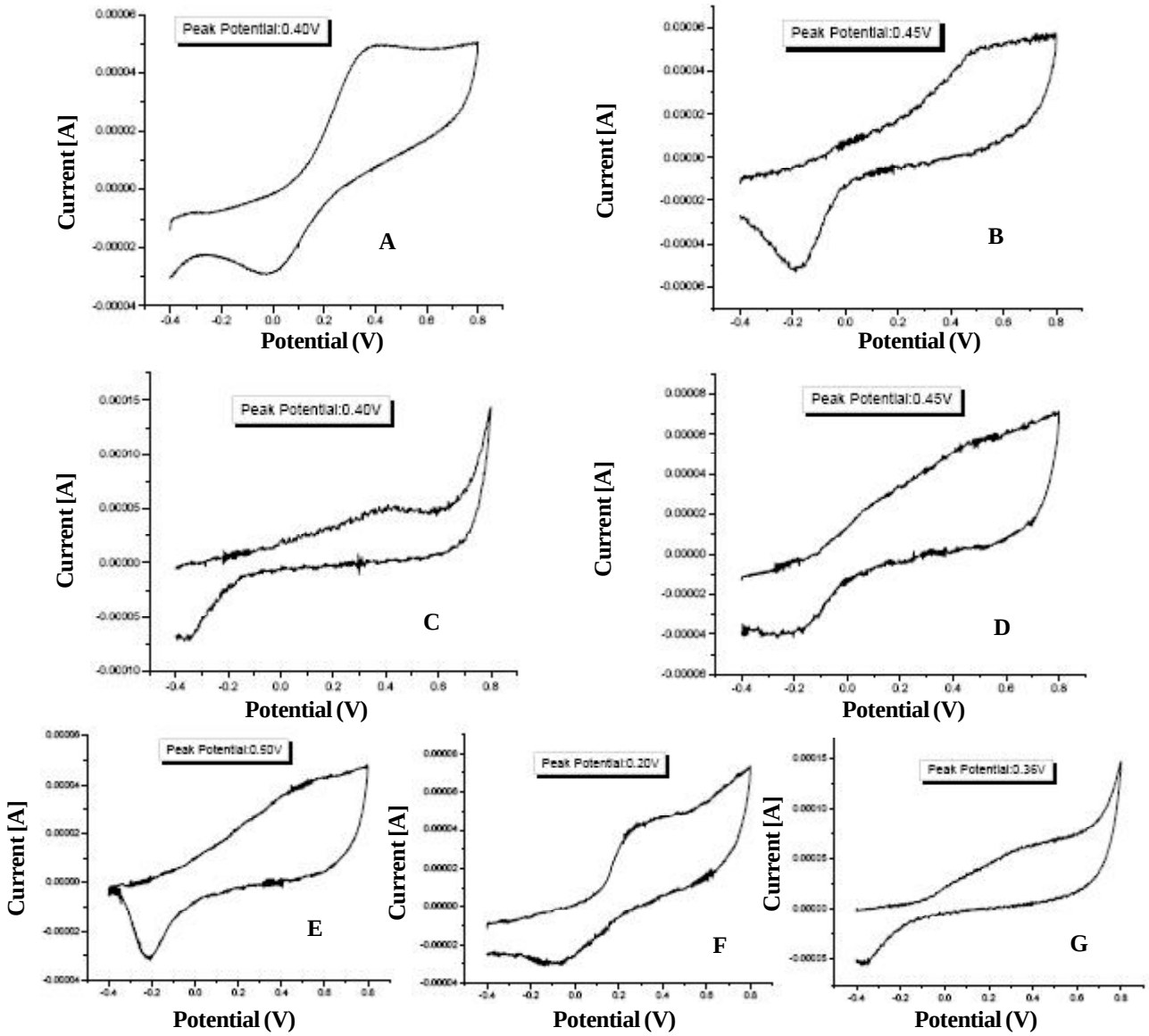

Figure 1: Representative cyclic voltammograms (at pH 11.2) for Vit. C and different plant extracts. A: Current –Voltage curve for Vit. C; B: Current –Voltage curve for *L.indica* EtOAc extract; C: Current –Voltage curve for *L.indica* $CHCl_3$ extract; D: Current –Voltage curve for *R.tetraphylla* fruits EtOH extract; E: Current–Voltage curve for *Z.diphylla* aqueous extract; F: Current –Voltage curve for *C. minima* EtOH extract; G: Current –Voltage curve for *C.minima* EtOAc extract.

oxidant potential in comparison to ascorbic acid (vitamin C) using cyclic voltammetric technique[9-13].

Cyclic voltammetric (CV) studies with the plant extracts were carried out to estimate the oxidisabilities (or the reduction potentials) of different plant extracts with varying concentrations; such studies are quite helpful to provide a criterion for the reversibility of redox reactions[14-18]. It is anticipated that a good reversibility in the CV curve could afford an idea about the potency in antioxidative efficacy of the experimental plant extracts.

EXPERIMENTALS

Preparation of plant extracts

The following plant extracts were prepared from air-dried and powdered plant materials in a Soxhlet apparatus using organic solvents of varying polarity. The extracts were then concentrated under reduced pressure using rotary evaporator.

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TABLE 1 : Estimation of vitamin C equivalent present in different plant extract

Sl. no.	Compound / extract of plants	Amount taken (g)	Peak potential (E_p)	Peak Area (V.A)	Estimated concentration (mM)	mM of Vit. C equiv./g of extract
1	Ascorbic acid (Vit.C)	0.142	0.40V	6.11×10^{-3}	0.806	–
2	*L.indica* EtOAc extract	0.098	0.45V	6.17×10^{-3}	0.813	8.30
3	*L.indica* $CHCl_3$ extract	0.058	0.40V	4.58×10^{-3}	0.697	12.01
4	*R.tetraphylla* fruits EtOH extract	0.063	0.45V	7.66×10^{-3}	1.385	21.90
5	*Z.diphylla* aqueous extract	0.103	0.50V	5.26×10^{-3}	0.604	5.83
6	*C.minima* EtOH extract	0.054	0.20V	7.18×10^{-3}	0.945	17.20
7	*C.minima* EtOAc extract	0.047	0.36V	1.05×10^{-2}	1.008	21.25

Limnophila indica (family: scrophulariaceae.)[4,5]

Ethyl acetate extract of *L.indica* **(B)** was shaken with petroleum ether and the residue was collected. The residue was dissolved in 5% NaOH solution and then neutralised by dilute HCl. The whole solution was concentrated to obtain the solid mass. Another extract of *L.indica* **(C)** were prepared using chloroform as solvent.

Rauwolfia tetraphylla (family: apocynaceae)[4,6]

The extract of *R.tetraphylla***(D)** fruits was prepared using cold ethanol as solvent. The concentrated mass was treated with dilute acetic acid and then neutralised by adding dilute NH_4OH solution. The residue was collected and dried to pasty mass.

Zornia diphylla (family: Fabaceae)[6]

The aqueous extract of *Z.diphylla* **(E)** was concentrated under reduced pressure to obtain the solid mass.

Centipeda minima (family: Asteraceae)[4,7,8]

The ethanol extract **(F)** and ethyl acetate extract (G) of *C.minima* were prepared using similar technique.

Cyclic voltammetric study

Cyclic voltammograms of the extracts were recorded with a potentiostat/Galvanostat (model-Versa Stat™ II, PAR) coupled to a computer software using a three electrode set up, where a glassy carbon serves as the working electrode (1.00 cm^2), Pt-wire as counter electrode and a saturated calomel electrode (SCE, 0.242V) as the reference electrode. The B-G pattern were recorded after dissolving the extract in an aqueous solution having pH 11.2 in the potential range of – 0.4 to +0.8 V vs. SCE at a scan rate of 50 mV/s. the amount of Vit. C equivalent in each plant extract was estimated using the following formula:

Concentration of vitamin C equivalent = Concentration of vitamin C×Peak area for extract /Peak area for vitamin C

RESULTS AND DISCUSSION

Cyclic Voltammetric method was used to estimate the total antioxidant capacity of some traditionally used medicinal plant extracts as well as ascorbic acid (vit. C). Current vs. Voltage curves for different plant extracts **(B-G)** were shown in figure 1. None of the extracts were found to show reversibility but broad peaks were observed at 0.45V, 0.40V, 0.45V, 0.50V, 0.20V and 0.36V respectively for **B**, **C**, **D**, **E**, **F** & **G**; this feature may be attributed to the presence of mixture of several oxidisable substances (presence of several polyphenolic compounds within the plants have been reported earlier[19-21]) into the extracts. The curve 'A' in figure 1 shows the current vs. voltage pattern of 0.806 mM solution of vitamin-C at pH 11.2. The observed peak maxima (E_p) is 0.40V vs. saturated calomel electrode as reference and the area (6.11×10^{-3}) under the peak has been calculated by integration method (TABLE 1), and this value was used as the reference to estimate the concentration of oxidisable natural chemical entities (as ascorbic acid equivalent) present in different plant extracts. The peak area for each of the extracts were measured using the same procedure and the data have been presented in TABLE 1. From the peak area and the peak current (TABLE 1) of the extracts as well as Vit. C, the concentration of oxidisable equivalence present in each extracts was determined by comparison method. This technique, thus, provides a way to estimate the amount of antioxidant compounds present in different plant extracts as shown in TABLE 1.

CONCLUSION

Cyclic voltammetry affords a sensitive method of determining concentration of easily oxidisable chemicals that may help in neutralizing the free radicals of several oxidants via electron transfer reaction[22-24]. A variety of natural compounds including polyphenolics are known to show antioxidant activity, mainly due to their redox properties[25-27]. The experimental results (viz. estimated concentration of Vitamin-C equivalent present in each plant extract) revealed that all the plant extracts as examined show antioxidant property comparable to vitamin-C. Hence, it may be anticipated that the plant extracts contain certain chemical entities responsible for their antioxidant efficacies. The present work would surely motivate the phytochemists as well as the pharmacologists to undertake in-depth studies on these useful plants.

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