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Assessment of Antioxidant and Antibacterial Properties of Ethnomedicinal Vegetables in North East India

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ABSTRACT

The study evaluated the antioxidant and antibacterial activities of three North East Indian ethnomedicinal vegetables viz., *Oxalis corniculata*, *Portulaca oleraceae*, and *Polygonum microcephalum* by cyclic voltammetry and agar well diffusion method respectively. The antioxidative effects have been evaluated by monitoring the change of the oxidation potential in the redox cycle of 1,4-diaminobenzene in presence of hexane, ethyl acetate and methanol extracts of the plants. 1,4-Diaminobenzene exhibits two reversible redox cycles with $E_{1/2}$ at 218 mV and 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, the oxidation waves were delayed and the radical cation was scavenged, and disappearance of the second oxidant waves in the cyclic voltammograms. Additionally, the methanol extracts demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria. These findings suggest the potential of these vegetables as natural antioxidants and antibacterial agents.

1. Introduction

Nutraceuticals and functional foods play a vital role in preventing and managing lifestyle-related diseases, such as diabetes, obesity, and heart disease. With the growing awareness of health-promoting ingredients, plant-based products have gained significant attention.

Recently, medicinal plants have emerged as a focal point of interest due to their rich phytochemical composition and potential preventive health benefits [1-3].

Now a days, medicinal plants are gaining increasing recognition due to the rising demand for their antioxidant properties. In the food industry, antioxidants are used to delay the oxidation process [4]. As a result, natural substances derived from medicinal plants are being explored as potential substitutes for synthetic antioxidants.

The antioxidant properties of biological samples, foods, extracts and pure substances were 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 CV was introduced relatively late in the study of antioxidant activity, research has revealed its unique advantages in investigating the antioxidant properties of polyphenols [3,7,8]. This is because polyphenols involve electron transfer, making CV an ideal technique for analysis. CV enables the determination of key parameters, including the oxidation potential of an analyte, the number of transferred electrons, and the rate of the electrode reaction.

Furthermore, CV has been successfully applied to evaluate the antioxidant properties of wine polyphenols, comparing the antioxidant activities of phenolic acids, flavonoids, and tocopherols [9] and the total antioxidant capacity of edible plants [10-12]. In earlier work we have reported the comparative study of antioxidant activities of some fresh and preserved herbal food products of North East India by CV [3]. Initially, CV measurements were used to assess the integrated antioxidant capacity, which is attributed to low molecular weight antioxidants (LMWA) [12]. However, these early measurements did not provide specific information on the contribution of individual antioxidant components. But a late, researchers have successfully used a combination of CV and spectroscopic

methods to quantify the specific contribution of quercetin and its glucosides to the antioxidant capacity of onions [13].

In the CV method, the reductive potential of a compound or a mixture is measured by its ability to donate electrons. This is because most of the LMWA act as reducing agents, neutralizing reactive oxygen species (ROS) by donating electrons.

As a result, CV has been employed to estimate the reduction potential of herbal extracts and detect the presence of oxidizable substrates within them [14]. CV can also be used to investigate the impact of a sample on a well-characterized redox system by monitoring changes in the system. For instance, CV was employed to study the effect of linear phenol-aldehyde condensation oligomers on the redox behavior of 1,4-diaminobenzene. The results showed that the oligomers delayed the oxidation process by stabilizing the system through hydrogen bonding or host-guest interactions [3, 15].

1,4-Diaminobenzene was selected for this study due to its well-defined reversible redox cycles, with half-wave potentials ($E_{1/2}$) at 218 mV and 535 mV in DMF with the oxidation waves which correspond to the formation of a radical cation and a diiminium dication, respectively.

The antioxidant properties of plant extracts can be assessed using CV, as any delay in oxidation processes and/or scavenging of radicals will be reflected in the CV tracing [16].

This study examined the changes in the redox behavior of 1,4-diaminobenzene when combined with various extracts from three leafy medicinal vegetables viz., *Oxalis corniculata* L. (*Oxalidaceae*), *Portulaca oleraceae* L. (*Portulacaceae*), and *Polygonum microcephalum* Don. (*Polygonaceae*) which are used by the indigenous people of North East India as additive vegetables in fresh form. Moreover, the plants *Oxalis corniculata* and *Portulaca oleraceae* are considered as good for stomach disorders and *Polygonum microcephalum* is given to patients suffering from measles/chicken pox in the healing stage.

2. Experimental Methods

2.1 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 Dhemaji College, Dhemaji, Assam. 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 desiccator.

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Table 1 Plant materials under investigation

S. No.	Plant names / Family	Parts used / Form of use	Method / Purpose of use
1	<i>Oxalic corniculata</i> L. [Oxalidaceae]	Whole plant	Used as additive in making curries and considered as good for stomach disorders.
2	<i>Portulaca oleraceae</i> L. [Portulacaceae]	Whole plant	Added to curries to give a sour and cooling taste. It is considered good for dysentery etc
3	<i>Polygonum microcephallum</i> Don. [Polygonaceae]	Tender shoots	Used as medicinal vegetables. Added to curries to give a sour and cooling taste. Given to patients suffering from measles/chicken pox in the healing stage. Reported to accelerate the healing process.

2.2 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 H₂O was treated with 2.1 mL of aqueous 70% HClO₄. As a result, insoluble perchlorate was formed which was filtered and washed with cold H₂O and dried. Recrystallization 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.

2.3 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.

2.4 Microorganisms and Media

Microorganisms used in this study consisted of three Gram positive organisms viz., *Enterococcus faecalis* (MTCC 2729), *Micrococcus luteus* (MTCC 1538) and *Proteus mirabilis* (MTCC 743) and two Gram negative organisms viz., *Escherichia coli* (MTCC 443) and *Enterobacter aerogenes* (MTCC 2822) were procured from Institute of Microbial Technology (IMTECH), Chandigarh. The antibacterial activity was determined by the Agar-well diffusion method.

2.5 Extraction, Fractionation and Concentration of Extracts

About 100 g of each of the dried plant material were made into powder form. The dried powder was extracted by a Soxhlet extraction apparatus first with hexane (Hex) and then with ethyl acetate (EtOAc) and methanol (MeOH), respectively taking about 400 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.

2.6 Electrochemical Measurements of Antioxidant Activity: Cyclic Voltammetry

The measurement was 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.

2.7 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 cm³) with 8 mg of TBAP as supporting electrolyte.

2.8 Recording of Cyclic Voltammogram of 1,4-Diaminobenzene in presence of 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.

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2.9 Antibacterial Activity by Agar Well Diffusion Method

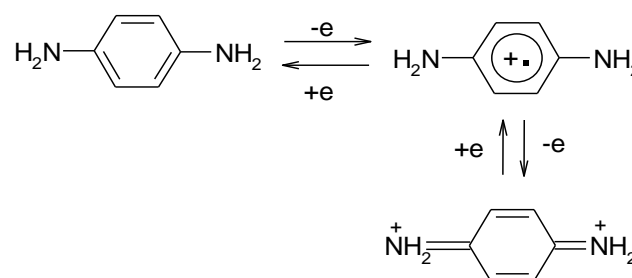
The antibacterial activity was determined by the agar-well diffusion method [17,18]. A 100 µL volume of the standard suspension (10⁹ CFU/mL) of each test bacterial strain was spread evenly on Nutrient Agar Petri plates (10 x 100 mm diameter) using sterile glass rod spreader and the plates were allowed to dry at room temperature. Subsequently, 6 mm-diameter wells were bored in the agar. A 100 µL volume of each plant extract, prepared in deionised water which showed no zone of inhibition and act as a negative control, were added into the wells by using 65 micropipettes.

Simultaneously the standard antibiotics (as positive control) were tested against the pathogens. Tetracycline (30 µg) and streptomycin (1 mg) were used as positive controls. All product of Himedia Laboratories Mumbai (India) were used in this study. Then the plates were incubated at 37 °C for 24 h. At the end of the incubated period, inhibition zones formed on the medium were evaluated in mm from the edge of zone to the edge of the well. Studies were performed in duplicate and the inhibition zones were compared with those of standard antibiotics.

3. Results and Discussion

3.1 Measurements of Antioxidant Activity: Cyclic Voltammetry

With cyclic voltammetry, the effect of plant extracts on the electrochemical behaviour of 1,4-diaminobenzene has been studied. As 1,4-Diaminobenzene is an amine with well-defined redox cycle (Scheme-1), so any change occurred to the redox behaviour may be studied conveniently by cyclic voltammetry. The 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.



Scheme 1 Redox cycle of 1,4-diaminobenzene

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 diamine.

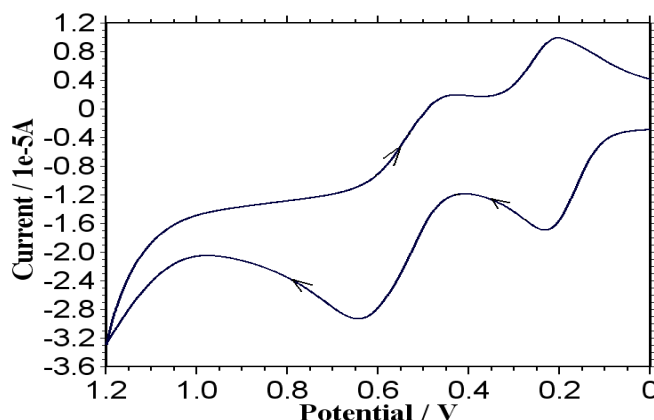


Fig. 1 Cyclic voltammogram of 1,4-diaminobenzene

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(a) – 2(c) and the shifts and/or absence of anodic potential of the oxidation waves of 1,4-diaminobenzene are summarized in Table 2.

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

S. No.	Plant extracts	1 st Peak E_p in mV	2 nd Peak E_p in mV
1	None	230	620
2	Oc (Hex)	236	711
3	Oc (EtOAc)	245	751
4	Oc (MeOH)	250	711
5	Po (Hex)	246	--
6	Po (EtOAc)	252	--
7	Po (MeOH)	254	--
8	Pm (Hex)	239	647
9	Pm (EtOAc)	241	646
10	Pm (MeOH)	245	652

Oc= *Oxalis corniculata*, Po= *Portulaca oleracea*, Pm= *Polygonum microcephallum*

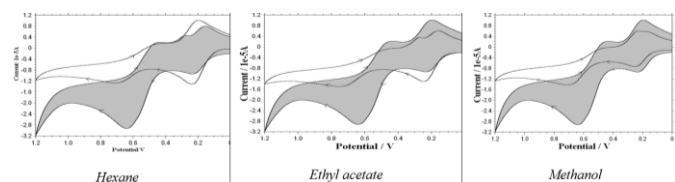


Fig. 2(a) CV of 1,4-diaminobenzene in presence of *Oxalis corniculata* extracts

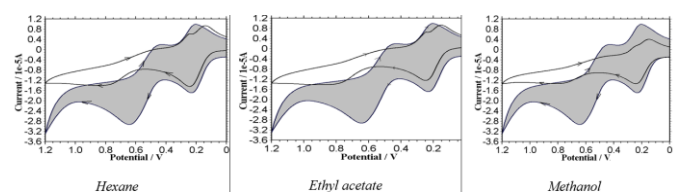


Fig. 2(b) CV of 1,4-diaminobenzene in presence of *Portulaca oleracea* extracts

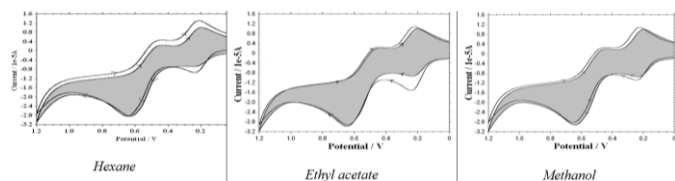


Fig. 2(c) CV of 1,4-diaminobenzene in presence of *Polygonum microcephallum* extracts

The effects of different extracts of all three plant species are shown in Figs. 2(a) - 2(c). 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. oleracea* the second redox cycle has disappeared. The disappearance of the second oxidation wave may be explained as follows. 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. However, in presence of the other extracts, the second redox cycle was also only delayed which implies that these extracts were good inhibitors of the oxidation process. The E_p values of the redox cycles of 1,4-diamino benzene and that of it in presence of different plant extracts are summarized in Table 2.

3.2 Antibacterial Activity

Literature revealed that the plants under investigation possess some medicinal properties which are used to treat some diseases and infections by the local people. 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 vegetables were 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.

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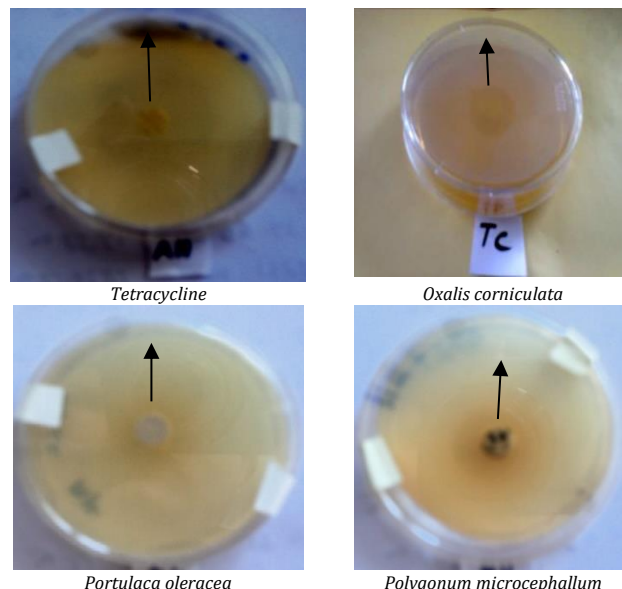


Fig. 3(a) Antibacterial activity against *Enterococcus faecalis*

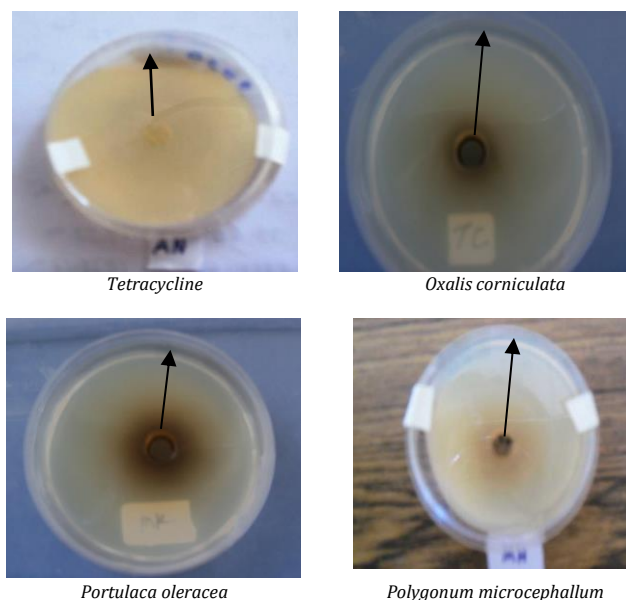


Fig. 3(b) Photos of antibacterial activity against *Enterobacter aerogenes*

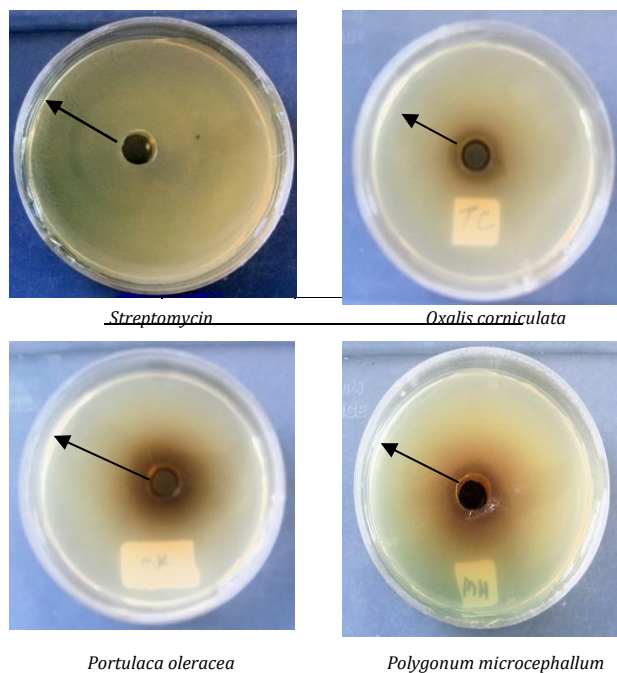


Fig. 3(c) Photos of antibacterial activity against *Escherichia coli*

3.3 *Micrococcus luteus* and *Proteus mirabilis*

The highest inhibition was found in *P. microcephallum* and *O. corniculata* 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.

Table 3 Zone of inhibition of methanol extracts of three medicinal vegetables

Bacterial species	Zone of inhibition (mm)			
	Antibiotic	<i>O. corniculata</i>	<i>P. microcephallum</i>	<i>P. oleracea</i>
<i>Enterococcus faecalis</i>	Tetracycline 11	8	6	8
<i>Micrococcus luteus</i>	Tetracycline 14	-	-	-
<i>Proteus mirabilis</i>	Tetracycline 13	-	-	-
<i>Enterobacter aerogenes</i>	Tetracycline 12	8	10	9
<i>Escherichia coli</i>	Streptomycin 11	7	8	7

4. Conclusion

The plant extracts under study showed that most of the samples significantly affected the overall redox reactions of 1,4-diaminobenzene. It is indicating the profound radical scavenging effect of the extracts. 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.

Additionally, the methanol extracts demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria. These findings suggest the potential of these vegetables as natural antioxidants and antibacterial agents. Furthermore, the methanol extracts exhibited notable antibacterial activity against a broad spectrum of bacteria, including both Gram-positive and Gram-negative strains. These results underscore the potential of these vegetables as valuable sources of natural antioxidants and antibacterial agents.

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