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Electrochemical Study of Aqueous Extract of Star-fruit

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Abstract

The electrochemical and biochemical study of water extract of Starfruit (*Averrhoa carambola L.*), was screened for antioxidant power and total phenolic content by rapid electrochemical technique where indium doped tin-oxide (ITO) glass was used as working electrode over the conventional glassy carbon elctrode. The chemical assays predicted the determination of the reducing power while electrochemical assays evaluated the presence of active electroactive organic compound by cyclic Voltammetry and differential pulse Voltammetry compare to standard, Gallic acid and ascorbic acid.

Keywords: Reducing properties, Cyclic voltammetry, ITO glass electrode.

Objective

The objective of this work to focus on rapid detection and sensitive analytical technique using commercially available ITO glass electrodes for antioxidant properties, e.g. reducing strength of food and natural products over the existing spectrochemical or other analytical techniques. Here, we can use native sample dissolve in a buffer at a constant pH at room temperature.

Introduction

The explore of natural and dietary antioxidants is emerging due to the concern of the public health for their safety, besides commercially available of synthetic analogues. Epidemiological evidence shows an association between decreased risk of cardiovascular disease and a diet rich in fruits and vegetables. Vegetables and fruits are also reported to decrease the risk of degenerative diseases and could have a protective effect against oxidative stress[1]. These effects may be related to natural antioxidants, including phenols, phytates, flavonoids, vitamin E and vitamin C which have the ability to scavenge free radicals and protect cells from the damage caused by free radicals.

Several strategies for analysis of the antioxidant activity (AA) of compounds are developed, like catalyst (or enzymatic) or non catalyst (or non-enzymatic) mensuration of lipid peroxidation inhibiting effects, active free radical scavenging capability and then on. It's clear that the information obtained by completely different assays replicate solely the precise inhibitor capability within the corresponding system. However it ought to be noted that inhibitor capability isn't obsessed with one straightforward specific reaction and

completely different mechanisms may be concerned with inhibitory activity. So as to determine the general inhibitor capability gift in food and medicinal plants, In order to evaluate the overall antioxidant capability present in food and medicinal plants, electrochemical approaches without the use of a certain reactive species could be applied[2].

Voltammetry is an increasingly popular electrochemical method applied to the determination of antioxidants in real samples[3], because it offers low detection limits, even when compared to more expensive techniques. It requires little or no time sample preparation. This technique provides us with the advantage of a fast analysis as well as with the easiness and the rapidity of the standard addition method application along with simplicity of the employed procedures, voltammetry appears to offer an attractive alternative to the titrimetric or instrumental methods mentioned earlier, in particular in food quality control. It does not require complicated, expensive equipment and well-qualified personnel nor is it laborious or time consuming like the conventional instrumental techniques[3,5,6]

The comparative analysis using cyclic voltammetric process and spectroscopic measurement for antioxidant activity measurement (Reducing Strength). This work focused that the CV method is very fast, cheap and sensitive analytical technique using ITO glass electrode over any other analytical or spectroscopic methods. Ascorbic acid (vitamin C) is a water-soluble vitamin which can be found in many biological systems and foodstuffs (fresh vegetables and fruits, namely, citrus)[1,8]. It plays an important role in collagen biosynthesis[6,11,12] iron absorption, and immune response activation and it is also acts as a powerful antioxidant which fights against free-radical induced diseases. It is used as a standard material.

Star fruit (*Averrhoa carambola L.*) is a good source of natural antioxidants and can effectively scavenge free radicals[4]. It is grown in the tropic and sub-tropic regions of the world. It is quite a popular fruit and largely planted in Southeast Asia and many other countries. It is usually consumed fresh or made into fruit juice or juice drinks.Star fruit is a good source of natural antioxidants like proanthocyanidins, (“)-epicatechin and vitamin C When star fruit is used to produce fruit drinks, normally only the juice will be used and the residue is often discarded as waste or used to produce low-value by-products. The intend of this work is to investigate a method for reducing activity determination of water extraction of Star-fruit by cyclic voltammetry and the developed method is applied to the determination of antioxidant activity in different samples, and the obtained results were compared with those obtained by a conventional spectroscopic method.

Materials and Methods

Materials: Star-fruit (purchased from local market), Folin-Ciocalteu (FC) reagent (Qualigens),Gallic acid (Sigma), Ascorbic acid, Potassium ferricyanide, Tricholoro-acetic acid (TCA), Sodium phosphate salts (Spectrochem), Methanol (Rankem, HPLC grade), Whattman filter-paper, Indium doped tin oxide (ITO) glass (Sigma).

Instruments: μ AUTOLAB (FRA2), Spectrophotometer (JASCO-V-630), Centrifuge (REMI-R25).

Extraction of star-fruit: The Star-fruit was sliced with knife and weighed 5gm from it. Then the sliced was extracted in mili-Q water for 3hrs at 50^0C. The solution was filtered through Whattman filter paper, and the filter was taken as crude water extract of star-fruit.

Estimation of phenolic compounds: Total phenol values are expressed in terms of gallic acid equivalent, which is a common reference compound[10]. The detection range for standardization was predicted to be between 0.2 mM to 2.0 M concentration, as the standard curve was prepared by 0,200,400,800,1000 μM solutions of gallic acid in 95% methanol (v/v).

The water extract samples (0.15 ml) were mixed with the Folin Ciocalteu reagent (300 ml, diluted to 10%, with distilled water) for 5mins and aqueous Na_2CO_3 (0.7 M, 1.2 ml per reaction mixture) were then added. Ratio for each reaction mixture should be 1:2:8 and each component was added in a proper sequence. The mixture was allowed to stand for an hour of incubation and the absorbance was determined by spectrophotometer at 765 nm.

Reducing activity measurement: The reducing power was measured by the method of Oyaizu (1986)[9], Water extracts of star fruit (2.5 ml) were mixed with sodium phosphate buffer (0.2 M, pH 6.5, 2.5 ml) and potassium ferricyanide [$K_3Fe(CN)_6$] (1%, 2.5 ml). The mixture was then incubated at 50 °C for 20 min. Tricloroacetic acid (10% w/v, 2.5 ml) was then added and the mixture was centrifuged at 3000 rpm for 10 min (REMI R25). From the supernatant layer (2.5 ml), 2.5 ml of deionised water and 0.5 ml of ferric chloride (0.1%) were added, and the absorbance was measured at 700nm. Higher absorbance indicates better reducing power. Ascorbic acid was used as standard.

Cyclic voltammetry: The working concentration of ascorbic acid 1mg/ml and for water extract of star fruit, 1.5mg/ml. Buffer solutions of 0.1M phosphoric acids, for pH 7, was used as electrolyte. A potentiostat-galvanostat cyclic voltammeter, μ AUTOLAB, Type III (FRA2) was used, as well as the respective software 'GPES' was used for the screening the output results. Cyclic voltammetric measurements were carried out using a conventional three electrode system in a single compartment cell. The three electrode system was composed of a working electrode and a reference electrode along with a counter one. In contrary to many other protocols, we usesd a glassy 'indium doped tin oxide'(ITO) as working one, in spite of glassy carbon electrodes[7]. For the rest an Ag/AgCl as reference electrode was used in conjunction with a platinum (Pt) counter electrode. Voltammetric signals were recorded at 25^0C with scan rate of 30mV/s.

Results and Discussion

Total phenol content: Total estimated phenolic content by FC-reagent in water extract of star-fruit was 300 mg/gm of dry weight of star-fruit.

Reducing strength: From Figure 1 it clearly assumed that the Reducing strength of water extract of star-fruit gradually increases with the concentration. Also indicated in the degree of reducing the strength of the extract is higher than standard ascorbic acid.

The electrochemical study of water extract of star-fruit was carried out by cyclic voltammograms. Figure 2 and Figure 3 show cyclic voltammograms (CVs) observed for Ascorbic acid as a standard and water extract of star fruits on ITO glass electrode in aqueous medium; the sweep rate used for this measurement was 30mV/s. Figure 4 shows the sensitivity of the electrode, ITO glass electrode. It was observed that both the standard compound about 1 (g and extracted material <1.5 (g , amount is just sufficient for electro analysis. The graph is presenting the current developed by different concentration of extracting materials. With an increase in concentration, the current is increased up to a certain concentration and after that the electrode was deposited with materials. Electrodes can be recycled using sonication in buffer and washing with ethanol. Thus, the technique is sensitive and cost effective. The electrochemical data recorded are listed in Table 1.

Table 1: Electrochemical parameters of the voltammograms recorded for compounds studied.

Parameter	$E_{1/2}$/V	Slope/mA $cm^{-2}mg^{-1}$ ml
Ascorbic acid	0.2	204.23
Star-fruit extract	0.6	0.53

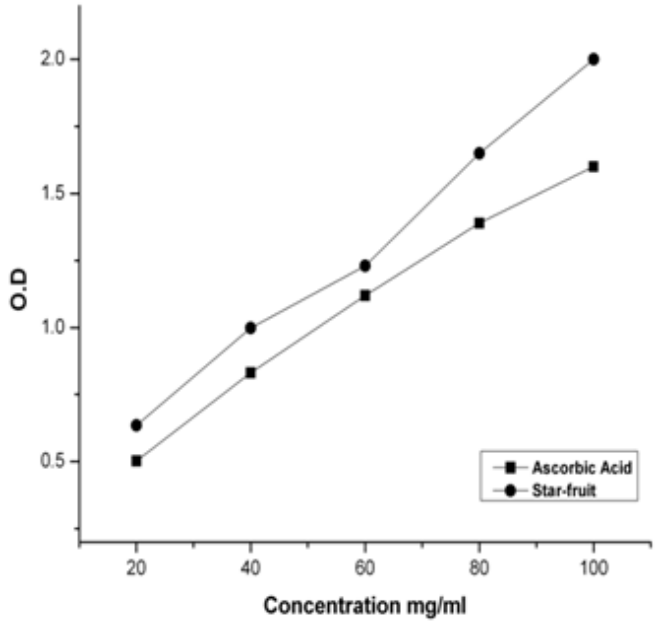

Fig. 1: Reducing strength of water extract of Star-fruit and Ascorbic acid in Spectroscopic assay**.**

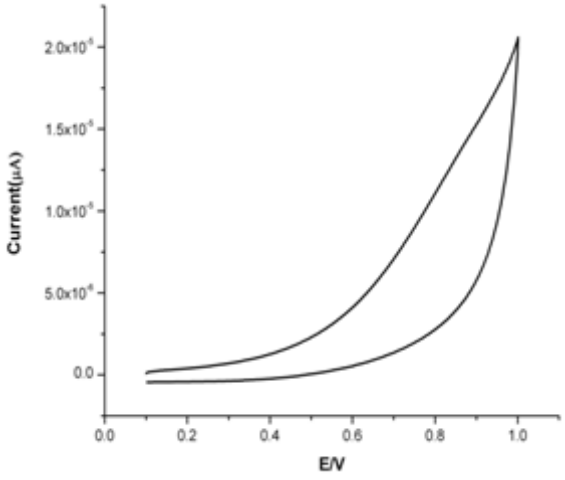

Fig. 2: Cyclic Voltagram of Ascorbic acid in 0.1M Phosphate buffer at pH 7

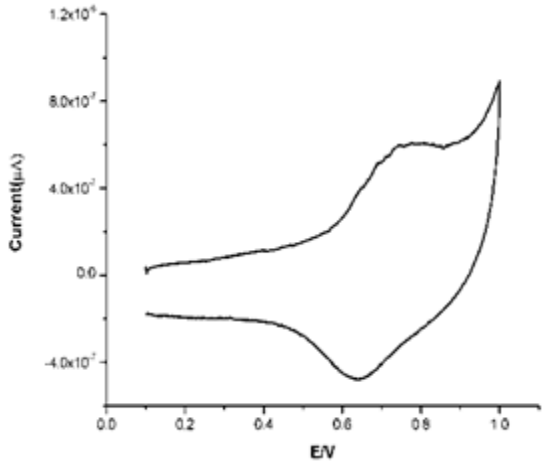

Fig. 3: Cyclic Voltagram of water extract of star fruits in 0.1M Phosphate buffer at pH 7

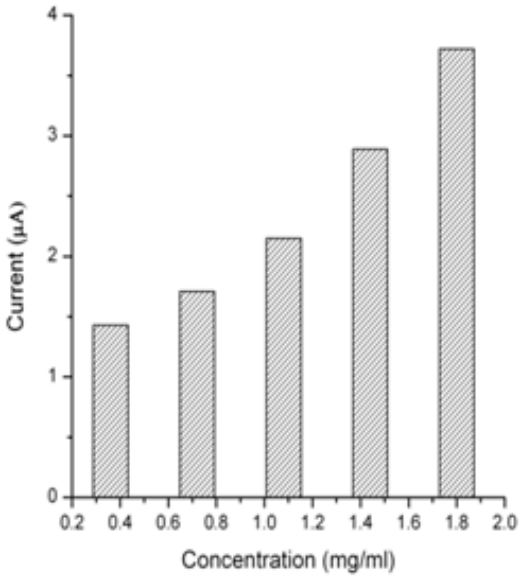

Fig. 4: Generation of current with different concentration of water extract of star fruit in 0.1M Phosphate buffer at pH 7 at 25°C.

Figure 5 shows the differential pulse Voltagram for water extract of star fruits and in increase in concentration the oxidation peaks are observed. Here the lower limit of detection of reducing strength is observed <1.5 (g dry weight of water extracted material.

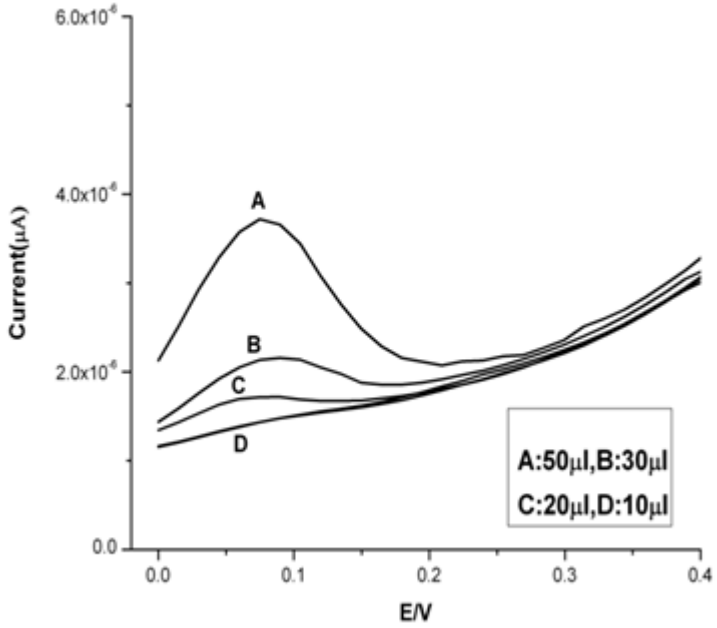

Fig. 5: Differential pulse voltagrams of Star-fruit water extract in 0.1M Phosphate buffer, pH 7.0.

Conclusion

In conclusion, our study showed that rapid detection of reducing strength of aqueous extract of star fruit as deduced from cyclic voltammetry analysis and it is correlated with spectrophotometric technique (Iron reducing assay) compared with standard and well known antioxidants. The electrochemical measurements present several advantages over spectroscopic measurements, the most significant being the electrochemical measurements can be carried out directly with water extract mixing with buffer at particular pH. It is a very rapid analysis technique (within 5min) and very minute amount of dry sample (for starfruit: < 1.5mg, and ascorbic acid : 1mg) is required for detection of reducing strength using ITO glass electrodes in phosphate buffer at pH 7. Also with compare to spectroscopic analysis, where required reagents are sometimes costly, this rapid electrochemical analysis is cost effective and the electrodes can be recycled after washing.

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