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Electrochemical evaluation of lipophilic antioxidants from *Iryanthera juruensis* fruits (Myristicaceae)

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Abstract: TLC autographic assays revealed in the hexane extract of *Iryanthera juruensis* (Myristicaceae) the presence of two compounds, with antioxidant properties towards β -carotene. They were isolated and identified as 3-methyl-sargachromenol (**1**) and sargachromenol (**2**). Further investigation of the hexane extract led to isolations of 3-methyl-sargaquinoic acid (**3**) and sargaquinoic acid (**4**). The electrochemical behaviour of these compounds was studied in $\text{CH}_2\text{Cl}_2/\text{Bu}_4\text{NBF}_4$ at glassy carbon electrode. The phenolic group in both tocotrienols **1** and **2** are oxidized at +0.23V and +0.32V and their oxidation potentials are correlated with the observed antioxidant activities and oxidation mechanism of α -tocopherol. The reductive voltametric behaviour of quinone function in both plastoquinones **3** and **4** is discussed.

Keywords: *Iryanthera juruensis*; antioxidants; cyclic voltammetry; Myristicaceae

Introduction

Iryanthera juruensis is a widespread amazonic species known as *ucu-úba rana* in Brazil, *mamita* in the Llanos of San Martin and *cuángare* on the Pacific coast. *Ucuúba* is the general designation given to amazonic myristicaceous species, whose seeds are valued in Brazil for their high yield in fatty acids, mainly myristic acid (C-14), which is extensively used in pharmaceutical and cosmetic industries. Other *Iryanthera* species were reported to have their crushed leaves used by Amazon Indians for healing seriously infected wounds and cuts and to have their latex from the bark mixed with warm water for treating stomachal infections[1]. Phytochemical work carried out on fruits of *Iryanthera grandis* and *I. lancifolia* disclosed the presence of tocotrienols which exhibited higher antioxidant activity than α -tocopherol, evaluated through malondialdehyde (MDA) and chemiluminescence (CL) assays[2]. Studies on tocotrienols biological activities revealed the ability of γ and δ -tocotrienol in inhibiting solid tumour growth of sarcome 180 and

also the highly metastatic Lewis lung carcinoma cells[3]. Moreover, these tocotrienols are effective against vascular smooth muscle cells proliferation, which plays a central role in atherosclerosis[4]. Oxidative damage to cell components is the initial event in several human disease processes including cancer and atherosclerosis[5], what has intensified the search for natural antioxidants.

The high yield of easily oxidizable fatty acids in myristicaceous fruits suggests the necessity of antioxidant substances being produced in the fruits in order to maintain the integrity of seeds and thereby increase the prospects of germination. Therefore, it was of interest to investigate the chemical composition of *I. juruensis* fruits in the search for new antioxidant substances[6,7].

The electrochemical behaviour of many simple phenolic compounds has been examined in the literature[8,9]. The existence of good correlation between the antioxidant activities and oxidation potentials for natural and synthetic compounds[10,11], explain the great interest to investigate the ox-redox properties of these classes

of compounds. Therefore, it is not surprising that vitamin E, as good antioxidant, have been subjected to several electrochemical studies[12-17].

In this work, the electrochemical oxidation of tocotrienols isolated from *I. juruensis* fruits was investigated at a glassy carbon electrode and its electrochemical behaviour compared to that of vitamin E (α -tocopherol) with the aim to evaluate its antioxidant properties. The advantages of electrochemistry have also been used to follow the generated intermediates and products by comparing the results of electrochemical reduction of plastoquinone isolated from the same fruit.

Experimental

Phytochemical procedures

Fruits were collected in Reserva do Mocambo, in the Amazon Forest, in the rain season. A voucher specimen is deposited at Museu Paraense Emilio Goeldi, in Belém, PA, Brazil. The dried fruits were grounded and extracted with hexane. The extract was dried under reduced pressure and partitioned between hexane and 10% aqueous methanol. The hydroalcoholic fraction (7.6 g) was submitted to vacuum column chromatography over silica gel, which was eluted with hexane/EtOAc mixtures of increasing polarities and yielded 8 fractions. Fractionation of the crude extract guided by the antioxidant TLC assay led to the choice of fractions B, C and K to be further investigated[18]. Flash column chromatography over silica gel of fraction B eluted with gradient of hexane/EtOAc afforded **2** (31 mg), **4** (53 mg) and others 13 fractions. Purification of fractions E and K through preparation of TLC (hexane/EtOAc 8:2) gave **3** (15 mg) and impure **1**, which was further purified by TLC (hexane/EtOAc 8:2) and afforded pure **1** (12 mg). The isolated metabolites were identified as 3-methyl-sargachromenol (**1**), sargachromenol (**2**), 3-methyl-sargaquinoic acid (**3**), sargaquinoic acid (**4**), respectively, from their spectroscopic data (IR, EI and ESMS, ^1H and ^{13}C -NMR)[18].

Apparatus

NMR spectra (^1H , ^{13}C , COSY and NOE experiments) were measured on a Bruker ACF-300 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C) in CDCl_3 for all compounds; the chemical shifts are reported in ppm with TMS as internal standard, and

coupling constants (J) are given in Hz. Electron impact spectra (70 eV) were recorded on a HP 5988-A.

β -carotene assay

After developing and drying, TLC plates were sprayed with a 0.02% solution of β -carotene (Aldrich), in CH_2Cl_2 . Plates were placed under natural light until discoloration of background. The remaining yellow spots indicated the presence of antioxidant substances.

Electrochemical assay

The cyclic voltamograms were carried out with a Polarecord E-506 (Metrohm) potentiostat. A three electrode system (663 VA, Metrohm) with a working electrode of glassy carbon (GC), a Ag/AgCl reference electrode and a carbon auxiliary electrode was used. Dichloromethane (Merck) was used as solvent and tetra-*n*-butyl ammonium tetrafluoroborate (Merck), as the supporting electrolyte. Vitamin E (Aldrich) was used as reference compound. The working electrode was polished with aluminium oxide 0.3 m (Buehler) and special polishing cloth (Buehler) prior to each measurement, because formation of a polymeric film inactivated the electrode after each scan. The electrode was then washed thoroughly with water, dried and cycled several times in the range -1.6 to $+0.6$ V in order to reassess the absence of voltametric signal due to the supporting electrolyte. Each compound was dissolved to concentrations of 10-20 mM in dichloromethane and added to a 15 mL aliquot of the supporting electrolyte. The solutions were then submitted to nitrogen flow during 10 min and the cyclic voltamograms were carried out at scan rates of 50 mV/s, unless stated otherwise.

Results and Discussion

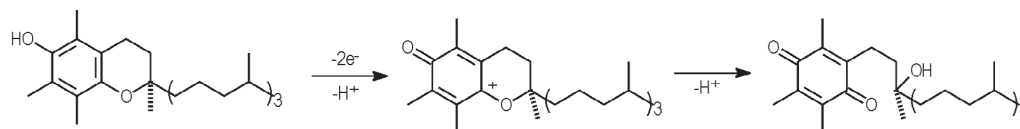
Chromatographic fractionation of the extracted deriving from fruits of *I. Juruensis* after isolation and identification based on spectrometric data (experimental section) leads to four main compounds assigned tocotrienols **1** and **2** and plastoquinones **3** and **4**[18], which chemical structures are proposed in Fig.1. Previous tests based on TLC analysis of these compounds using β -carotene solutions revealed the presence of antioxidant properties for tocotrienol **1** and **2**. In order to confirm its antioxidant properties voltammetric studies were

carried out for both isolated compounds and also for plastoquinones **3** and **4**, respectively.

Cyclic voltammograms obtained for oxidation of tocotrienols **1** and **2** in $\text{CH}_2\text{Cl}_2/0.1 \text{ M Bu}_4\text{NBF}_4$ at glassy carbon electrode presented only one anodic at +0.23V and +0.32V, respectively, as shown in Figure 2 (Curve A and C). The absence of cathodic peak in the reversal potential scan was observed for tocotrienol **2** in all scan rates investigated, but tocotrienol **1** presented a small cathodic peak in the reverse scan. The intensity of this cathodic peak increases with scan rate but is always smaller than unity ($i_{pa}/i_{pc}=0.3$). In addition, both oxidation waves were displaced to more negative potentials and the current function ($i_p \cdot v^{-1/2}$) decreases when the scan rate increases, showing characteristics of eletroodic process involving a chemical reaction subsequent to the electron transfer[19]. Such behavior is indicative that probably the oxidation process involves the phenolic group in the tocotrienol compounds, but the subsequent chemical reaction consuming the generated product is more rapid for tocotrienol **2**.

In order to test the voltammetric results as a tool to evaluate the antioxidant capacity, the results were compared investigating the electrochemical behavior of α -tocopherol, with similar molecular structure of the tocotrienols isolated from *I. Juruensis*. Typical cyclic voltammograms of α -tocopherol exhibits only one well-defined anodic peak at +0.28 V (peak I_a) and one cathodic peak in the reversal potential scan, as shown in Figure 2, Curve B. The anodic peak potential (peak I_a) is shifted to more negative values and the ratio of i_{pa}/i_{pc} increases from 0.3 to 0.8 when the scan rate is increased from 0.01 V s^{-1} to 2 V s^{-1} , suggesting that the product generated is also consumed by a slow chemical reaction[19]. This voltammetric behavior agree well with the global mechanism proposed for oxidation of the phenol group in α -tocopherol in aprotic medium[15-18], as shown in Scheme 1[18]. The oxidation of phenol group in the α -tocopherol occurs via two electron transfer and liberation of two H^+ . The consequent formation of the correspondent quinone is observed only after the ring cleavage occurring after an intermediate stage of charge stabilization.

Scheme 1. Electrochemical Oxidation of α -tocopherol.



Thus, taking into consideration the similarity between the molecular structures from tocotrienols **1** and **2** and α -tocopherol, it is possible to conclude that all the compounds are oxidized after complete conversion of the phenol group to the respective quinone, but can involve the formation of a stable cation as intermediate before oxidative cleavage. Certainly, this intermediate is responsible for the appearance of the cathodic peak observed in the reverse scan. Its absence in cyclic voltammograms of tocotrienol **2** indicates that the intermediate is less stable than tocotrienol **1** due successive cleavage of the C-O bond¹⁹. The respective values of peak potential calculated from cyclic voltammograms of all the compounds are compared in Table 1.

Table 1. Cyclic voltametric parameters of isolated compounds **1-4** from *I. juruensis* fruits and reference compound α -tocopherol in $\text{CH}_2\text{Cl}_2/\text{Bu}_4\text{NBF}_4$ on glassy carbon electrode, at scan rate of 50 mV/s .

| Substances | Oxidation | Reduction |
|----------------------|--------------|--------------|
| | E_{pa} (V) | E_{pc} (V) |
| α -tocopherol | + 0,276 | - 0,90* |
| 1 | + 0,233 | - 0,43* |
| 2 | + 0,322 | - 0,38* |
| 3 | - | -0.50 |
| 4 | - | -0.38 |

* E_{pc} refers to reduction potentials of the oxidized products of α -tocopherol and tocotrienols **1** and **2**, generated on the electrode surface.

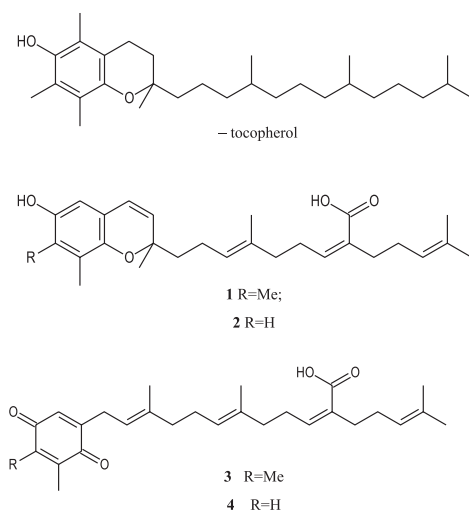


Figure 1. Chemical Structures of α -tocopherol, tocotrienols **1** and **2** and plastoquinones **3** and **4**.

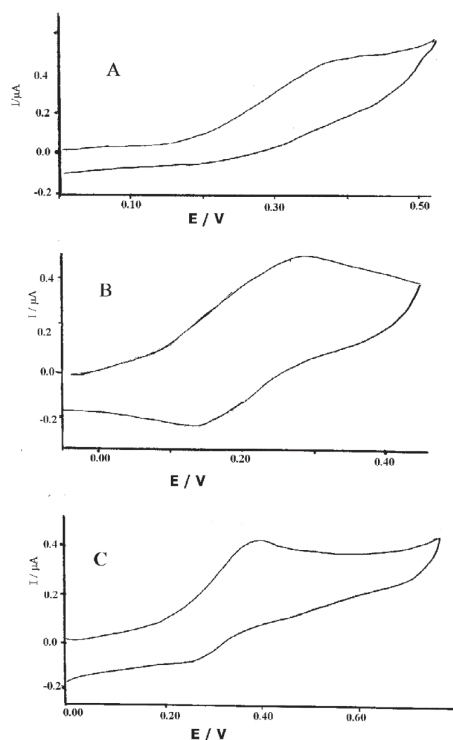


Figure 2. Cyclic voltammograms obtained for oxidation of 1.0 mmol dm^{-3} α -tocopherol (B); tocotrienol **2** (A) and tocotrienol **1** (C) in Bu_4NBF_4 (Curve B) at vitreous carbon run at 0.10V.

As expected, cyclic voltammograms recorded for newly prepared solution of tocopherols and tocotrienols do not show any reductive peak (Fig. 3, Curve A). Nevertheless, cyclic voltammograms obtained for the product generated after previous oxidation at +0.50V presents a well-defined peak at -0.43V (tocotrienol **1**) and -0.38V (tocotrienol **2**). Representative cyclic voltammograms for reduction of tocotrienol **2** is shown in Fig. 4. The behavior is very similar to that verified for reduction of the product generated previously during oxidation of α -tocopherol, as can be seen in Fig. 3. Table 1, summarize the oxidation potential and the correspondent potential where reduction of the main product generated (after oxidation at +0.50 V) is taking place for both tocotrienols and α -tocopherol. The occurrence of reduction step in the cyclic voltammograms recorded from 0 to -1.5V revealed that reduction of quinone or stable intermediate could be taking place within the scan time scale.

In order to test this hypothesis, complete cyclic voltammograms were recorded for plastoquinones **3** and **4** in $\text{CH}_2\text{Cl}_2/0.1 \text{ M TBATFB}$ at glassy carbon electrode, which were isolated and identified previously from *I. Juruensis* (experimental section). Figure 5 exhibits a cyclic voltammogram for oxidation and reduction of plastoquinone **4**. There is no oxidation peak for plastoquinone, but both compounds are promptly reduced at potential of -0.38V and -0.50V , respectively (Table 1). The plastoquinone **3** presents similar voltammetric behavior to that of Figure 5 and as verified for others tocotrienols reduction[17], a shoulder at more negative potential are also seen in the voltammograms but they were not analysed here. In both cases, the electrochemical behavior of plastoquinones resembles a global electrochemical process, expected when a simple quinone is reduced in aprotic solvent[17]. The analysis of shape and position of the peak potential on the cyclic voltammetric curves corresponding to the reduction of quinones **3** and **4** and the tocotrienols **1** and **2**, suggests that the products generated after oxidation of tocotrienols are structurally closely related to that verified for the isolated quinones.

So, the comparison among oxidation potentials of tocotrienols **1** and **2** and α -tocopherol (Table 1), indicates that tocotrienol **1** is the compound more easily oxidizable in relation to tocotrienol **2** and α -tocopherol. This is explained,

because alkyl substituents in the *meta* position to the aromatic hydroxyl have little effect on the ease of oxidation of phenolic compounds, but inductive effects from *ortho* or *para* alkyl groups may lower the oxidation potential and make the molecule even easier oxidized than the phenol itself[19-20]. This could explain the lower oxidation potential of **1** when compared to **2**, as they possess the aromatic substitution pattern of γ and δ -tocotrienol respectively. The lower oxidation potential of **1** when compared to α -tocopherol might be due to the C-3 double bond, which is conjugated with the aromatic ring and stabilizes the intermediate cation previous to completion of oxidation steps.

The analysis of the structural effects occurring in this case indicates, that the stabilizing effect of C-3 double bond on the intermediate cation due to the presence of one aromatic methyl group *ortho* to the hydroxyl (e.g. compound **1**) overcomes, although slightly, the stabilizing effect of two aromatic methyl groups *ortho* to the hydroxyl if the C-3 double bond is absent (e.g. α -tocopherol). The contribution

of this double bond is, however, not enough to keep up the intermediate cation stability if no methyl group *ortho* to the hydroxyl is present in the molecule (e.g. compound **2**). On the other hand, the analysis of the oxidation electrodic process shows that more unstable intermediates are observed for tocotrienols **1** and **2** in comparison with the reversibility for the α -tocopherol oxidation, which could suggest a strong participation of the pyrane ring double bond in the cleavage rate of the oxidized molecule.

The comparison of the reductive processes concerning the previously oxidized forms of **1**, **2** and quinones **3** and **4** suggests that the products generated after electrochemical oxidation of tocotrienols **1** and **2** are very similar to that identified as quinone **3** and **4** respectively, directly isolated from *I. juruensis* fruits.

These results are in agreement with the qualitative analyses of the antioxidative properties of compounds **1**, **2**, **3** and **4** through the β -carotene assay that showed similar intensities of spots only for the active compounds **1** and **2**.

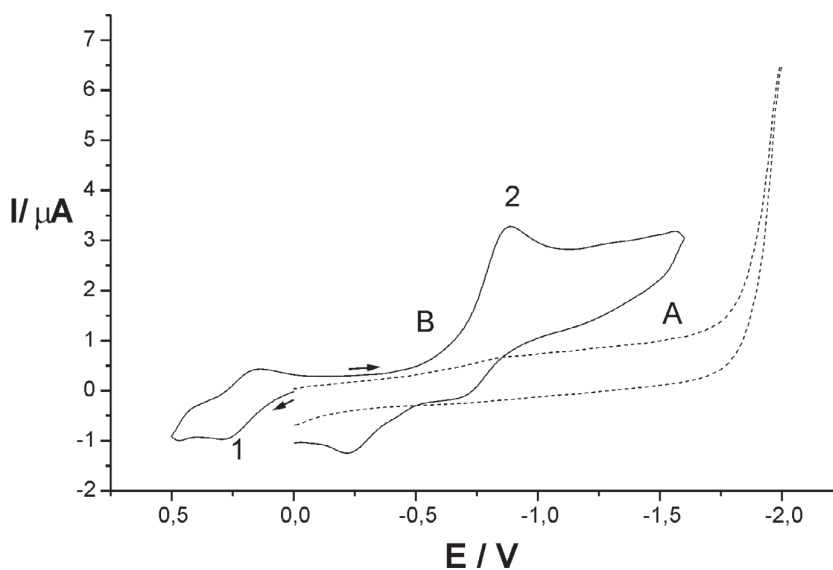


Figure 3. Cyclic voltammograms obtained for reduction of 1.0 mmol dm^{-3} α -tocopherol in Bu_4NBF_4 at vitreous carbon run at 0.10 V s^{-1} from 0 to -2.0 V (Curve A) and after previous oxidation from 0 to $+0.5 \text{ V}$ and reduction to -1.5 V (Curve B).

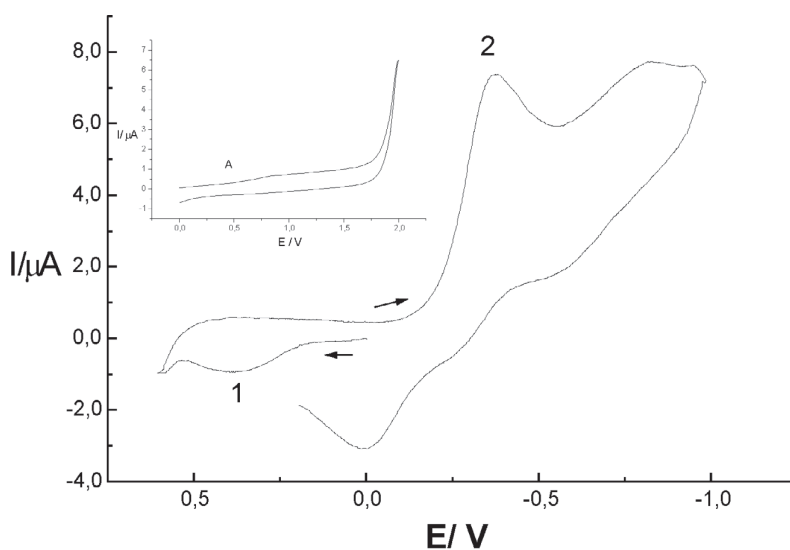


Figure 4. Cyclic voltammograms obtained for reduction of 1.0 mmol dm^{-3} tocotrienol 2 in Bu_4NBF_4 at vitreous carbon run at 0.10 V s^{-1} after previous oxidation from 0 to +0.5 V and reduction to -1.5V. Inlet: Supporting electrolyte.

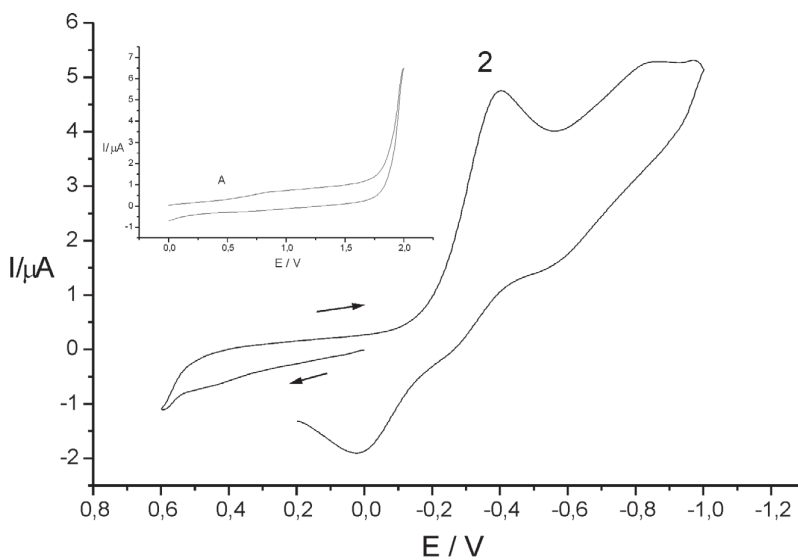


Figure 5. Cyclic voltammogram at vitreous carbon run at 0.10 V s^{-1} for oxidation followed by reduction of plastoquinone 4. Inlet: Supporting electrolyte.

Conclusions

From these studies, it was concluded that the antioxidant activity of a phenolic compound depends ultimately on its ease of oxidation. So, cyclic voltammetry could be a rapid and simple instrumental tool for evaluating the antioxidant capacity of phenolic derivatives in natural products. The comparison between the voltammetric behavior of vitamin E and tocotrienol extracted from *I. Juruensis* fruits have shown good correlations between antioxidant activity and low oxidation potential. As expected, the antioxidant activity is inversely

proportional to their oxidation potential, but other properties as chelating characteristics or enhancement of the radical stabilization after the initial oxidation steps seems also important in the process.

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D. H. S. Silva, F. C. Pereira, M. Yoshida, M. V. B. Zanoni. Avaliação do comportamento eletroquímico de antioxidantes extraídos dos frutos da *Iryanthera juruensis* (Myristicaceae)

Resumo: Testes em placas de CCDC reveladas com solução de β -caroteno mostraram a presença de duas substâncias com propriedades antioxidantes no extrato hexânico. Elas foram isoladas e identificadas como 3-metil-sargacromenol (**1**) and sargacromenol (**2**). O extrato hexânico forneceu ainda o ácido 3-metil-sargaquinóico (**3**) e o ácido sargaquinóico (**4**). O comportamento eletroquímico destas substâncias foi investigado em $\text{CH}_2\text{Cl}_2/\text{Bu}_4\text{NBF}_4$ sobre eletrodo de carbono vítreo. A oxidação do grupo fenólico nos tocotrienóis **1** and **2** é responsável por um pico anódico em potenciais de +0.23V e +0.32V, os quais são correlacionados com sua atividade antioxidante. O mecanismo de oxidação é comparado com o comportamento eletroquímico do antioxidante α -tocopherol.

Palavras-chave: *Iryanthera juruensis*; antioxidantes; voltametria cíclica; Myristicaceae.

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