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Electrochemistry-based chemotaxonomy in plants using the voltammetry of microparticles methodology

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Abstract A methodology for characterizing vegetal taxonomic groups using microextraction-assisted voltammetry of microparticles is described. It is based on recording the voltammetric response of microparticulate films of polyphenolic compounds of leaf extracts using different organic solvents. As a result, characteristic voltammetric profiles, tentatively defining an electrochemolomic response, are obtained. Bivariant and multivariant chemometric evaluation of the voltammetric responses of such films allows characterizing vegetal families. Analysis of voltammetric responses for a set of species of the Rosales order suggests that electrochemical data can be correlated with phylogenetic trees.

1. Introduction

Classification of plants is an essential task in botany, traditionally based on morphological/functional features. Nowadays, classification can be carried out by genetic analysis, typically identifying nucleotide sequences of nuclear and chloroplast regions.[1] In several cases, where chimaeric sequences were produced, there are discrepancies between molecular and morphological/functional classifications. This happens in the case of families like Guamatelaceae[2] or Hydatellaceae[3] as well as the so-called Rosid puzzle.[4,5]

Chemotaxonomy, that is, a method of biological classification based on the chemical composition of living organisms[6] has received considerable attention in the botanic domain, as recently reviewed.[7] Chemical analysis of the composition of plants and plant extracts has been extensively used to determine their nutritional, pharmacological and chemoecological properties. Among the plethora of components in vegetal matter, some bioactive phenolic components, such as anthocyanins, flavonols, procyanidins, and phenolic acids have been intensively studied because of their taxonomic significance.[8,9] In particular, the composition of plant cuticular waxes has been used for chemotaxonomic purposes because their composition varies sharply from one species to another, so that several compounds are present, in some taxa only in trace amounts and in others dominating the mixture.[10] Such wax extracts consist of homologous series of very-long-chain aliphatics (fatty acids, aldehydes, primary and secondary alcohols, ketones, and alkanes of chain lengths C20-C36, as well as C38-C70 alkyl esters, as well as triterpenoids, tocopherols, or aromatic compounds) whose identification, which involves chromatographic

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separations coupled to mass spectrometry detectors, is in general a complex analytical task.[11]

Among these components, natural antioxidants have received considerable attention because of their therapeutic capabilities,[12] derived from their antioxidant and radical scavenger capacities.[13-15] As far as the composition of plants is directly derived from their genetic profile, chemotaxonomic location of the different species is obvious interest in the search of natural products and synthetic analogues having specific therapeutic capabilities.[10,11]

In this context, electrochemical methods, all involving conventional solution-phase electrochemistry, have been proposed for evaluating the antioxidant capacity of natural products.[16-18] Additionally, the voltammetry of immobilized particles (VIMP) methodology has been applied for testing the antioxidative capacity of samples from fruits and vegetables.[19,20] The VIMP, a solid-state electrochemical technique which provides analytical information on sparingly soluble solids,[21-23] was previously used to distinguish indigo pigments prepared from different plants,[24] screening tomato fruit[25] and tea varieties.[26]

Here, we report a simple electrochemical methodology for obtaining taxa-characteristic voltammetric profiles aimed to provide a 'electrochemotaxonomic' approach which complement the existing chemotaxonomic techniques. The method is based on recording the electrochemical response of the polyphenolic components of leaf extracts with different organic solvents. The described method consists of a microextraction-assisted VIMP assay based on the voltammetric response of a microparticulate film of the components of the leaf extracts in contact with aqueous electrolytes. The proposed methodology exploits the voltammetric response of electroactive compounds in leaf extracts, representative of the plant composition,[27-30] leading to the characterization of vegetal taxonomic groups using chemometric analysis of the recorded voltammetric patterns. This technique provides several potential advantages over conventional analytical methods: (i) the representativity of the analytical data, ensured by the extraction procedure because the plant extracts are not exposed to prolonged aerobic oxidation; (ii) the relative simplicity of the voltammetric response, because many of the chemical components of the plant extracts are non-electroactive.

Voltammetric analysis has been applied here to 37 Rosid species representative of different orders and families (see Table 1). Nomenclature used was according to The Plant List (2013).[31] Voltammetric data of leaf extracts were correlated, via application of chemometric techniques, with phylogenetic data for the order of Rosales. This order is one of which include several uncertain phylogenetic relationships, sensu APG III (2009).[1] As reviewed recently by Baas et al.,[32] Sytsma et al.,[33] and Wang et al., 2009,[34] its families are morphologically heterogeneous[35] and had been considered to be distantly related and placed in different orders in traditional classifications[36,37] and genetic studies.[38,39] Here, it has been tested the correlation between the electrochemical pattern of species of the Moraceae, Urticaceae, Cannabaceae, Ulmaceae, Eleagnaceae, Rhamnaceae and Rosaceae families (all cosmopolitan families widely distributed in Europe) and the corresponding phylogenetic tree.[1] Our data suggest that there is possibility of acquiring information relevant for phylogenetic purposes from electrochemolomic data.

2. Experimental

Field studies were performed in the Botanic Garden of the University of Valencia in Valencia (Spain) during 2014. Entire leaves of adult plants were collected in September and October 2014. Adult specimens which were otherwise untouched and not submitted to cuts with an agricultural cutting machine were selected. All samples were placed between filter paper and stored in a desiccator at room temperature (298 K) for at least 24 h prior to electrochemical experiments. Unless stated, reported data correspond to electrochemical measurements performed 24 h after harvesting.

Electrochemical experiments were performed at 298 ±1 K in a CH cell using either a laboratory CH I660 potentiostat (Cambria Scientific, Llwynhendy, Llanelli UK) or Ivium CompactStat portable equipment (Ivium Technol. B.V., Eindhoven, The Netherlands). A BAS MF2012 glassy carbon working electrode (GCE) (geometrical area 0.071 cm^2), a platinum wire auxiliary electrode and an Ag/AgCl (3M NaCl) reference electrode were used in a typical three-electrode arrangement. Voltammetric measurements were performed with a freshly-prepared sample-modified GCE using 0.25 M aqueous acetic acid/sodium acetate buffer at pH 4.75 and 0.10 M potassium phosphate buffer at pH 7.0 as supporting electrolytes. For electrode preparation, ca. 500 mg of leaves were crashed with an agate mortar and pestle adding 0.5 mL of ethanol, acetone or chloroform (HPLC grade, Carlo Erba reagents) during 1 min 50 μL of the resulting suspension were dropped onto the GCE surface. After solvent evaporation in air, the electrode was inserted into the electrochemical cell and electrochemical runs were performed. SECM experiments were performed with CH 920c equipment using a microdisk platinum electrode tip (CH 49, diameter 20 μm) and a Pt substrate electrode (geometrical area 0.018 cm^2). The bipotentiostat mode was used to apply potentials to the tip (E_T) and the electrode substrate (E_S). The rate of scanning of the tip over the substrate was 20 μm/s for all experiments. The distance between tip and substrate was of the order of the tip electrode radius. A 2.0 mM solution of $K_4Fe(CN)_6$ in 0.25 M acetic acid/sodium acetate aqueous buffer at pH 4.75 was used as a redox probe in such experiments. Principal Components Analysis (PCA) was applied to voltammetric data using MINITAB14 software package.

3. Results and discussion

3.1. General voltammetric pattern

In contact with aqueous buffers, the microparticulate film of ethanolic extract of plant leaves displayed well-defined cyclic voltammetric responses (see Supplementary material, Fig. S.1). The more specific response was recorded in the region of potentials between −0.25 and +1.25 V vs. Ag/AgCl leaf extracts where, as shown Figure 1 for *Eonymus japonicus*, *Lobularia maritima*, *Koelrenteria bipinnata* and *Begonia acerifolia* in contact with aqueous acetate buffer, a series of overlapping anodic peaks appear defining clearly different voltammetric patterns. These signals can be attributed to the oxidation of polyphenolic compounds, as judged from a comparison with the voltammetric responses of rutin, quercetin, morin and other polyphenolic compounds in solution phase[40-44] and forming films[45,46] or microparticulate solids.[25,26]

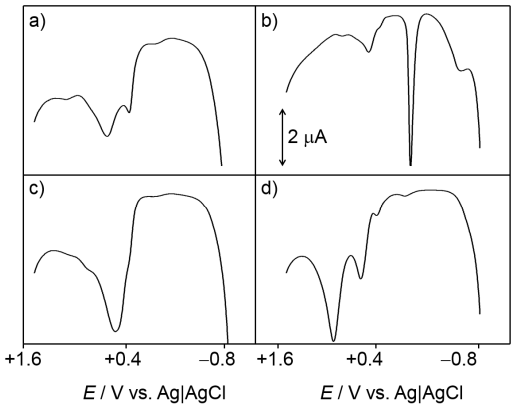

Figure 1. Square wave voltammograms of microparticulate films deposited on GCE of ethanolic extracts of leaves of: a) *Eonymus japonicus*, b) *Lobularia maritima*, c) *Koelrenteria bipinnata* and d) *Begonia acerifolia* in contact with 0.25 M aqueous acetate buffer, pH 4.75. Potential scan initiated at −1.05 V in the positive direction; potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz.

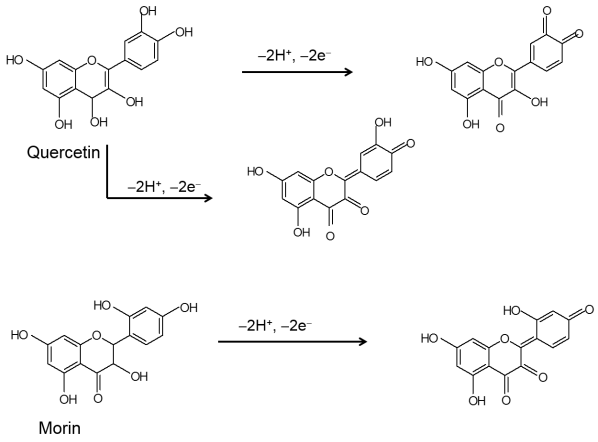

Figure 2. Scheme for the main electrochemical oxidation processes experienced by quercetin and morin.

In the case of quercetin, schematically illustrated in Figure 2, the main oxidation process consists of the two-electron, two-proton oxidation of the 3',4'-dihydroxybenzoic acid moiety yielding the corresponding *o*-quinone. This process can be accompanied by the oxidation of one of the –OH groups of the ring B coupled with that of the –OH bound to the C3 carbon in the ring C and the oxidation of the 5,7-dihydroxyl groups, occurring at higher potentials.[42] This would be the main oxidation process in cases such as morin, also schematized in Figure 2. The electrochemical processes involve mainly solid state reactions, (as confirmed by the persistence of the voltammetric response when the time of contact of the deposit with the electrolyte is prolonged) where electron ingress/issue is coupled, by reasons of charge conservation, to the entrance/issue of protons on/from the solid lattice, in agreement with extensive literature on the VIMP of organic compounds.[21-26] The involvement of protonation/deprotonation processes in the rate-determining step of most involved electrochemical processes results in the variation of peak potentials on the pH, in much cases fitting to a variation of 59 mV by pH unit corresponding to reversible n-proton, n-electron processes.[40-44]

Table 1. Rosid taxa used in this study. Numbers in parentheses corresponds to the species appearing in the dendrogram from Fig. S.6.

Order	Family	Species
Cucurbitales	Begoniaceae	*Begonia acerifolia* Kunth (**1**)
Fagales	Fagaceae	*Fagus sylvatica* L. (**3**), *Quercus macrocarpa* Michx. (**2**)
Rosales	Cannabaceae Eleagnaceae Moraceae Rhamnaceae Rosaceae Ulmaceae Urticaceae	*Celtis australis* L., *Humulus lupulus* L., *Trema orientalis* (L.) Blume *Elaeagnus × submacrophylla* Servett. *Ficus obscura* Blume, *Maclura pomifera* (Raf.) C.K. Schneid. (**4**) *Paliurus spina-christi* Mill., *Rhamnus alaternus* L. (**5**), *Ziziphus jujuba* Mill. *Potentilla reptans* L. (**8**), *Rosa canina* L. (**7**), *Rubus ulmifolius* Schott (**6**) *Zelkova carpinifolia* (Pall.) K. Koch *Boehmeria cylindrica* (L.) Sw., *Parietaria judaica* L. (**9**), *Urtica dioica* L.
Fabales	Fabaceae Quillajaceae	*Dorycnium pentaphyllum* Scop. (**12**), *Genista tinctoria* L. (**10**) *Quillaja saponaria* Molina (**11**)
Celastrales	Celatraceae	*Euonymus japonicus* Thunb. (**13**)
Malpighiales	Euphorbiaceae Violaceae	*Ricinus communis* L. (**14**) *Viola odorata* L. (**15**)
Malvales	Cistaceae Malvaceae Passifloraceae	*Fumana ericifolia* Wallr. (**18**) *Althaea officinalis* L. (**16**) *Passiflora edulis* Sims (**17**)
Brassicales	Brassicaceae	*Isatis tinctoria* L. (**25**), *Lobularia maritima* (L.) Desv. (**26**)
Sapindales	Anacardiaceae Rutaceae Sapindaceae	*Pistacia lentiscus* L. (**19**) *Ruta chalepensis* L. (**20**) *Koelreuteria bipinnata* Franch. (**21**)
Myrtales	Myrtaceae	*Myrtus communis* L. (**22**)
Geraniales	Geraniaceae Oxalidaceae	*Pelargonium capitatum* L'Hér. (**24**) *Oxalis articulata* Savigny (**23**)

The solid state nature of the involved electrochemical processes was assessed by SECM. Films of ethanolic and acetone extracts were prepared on a Pt substrate electrode and placed in contact to a $K_4Fe(CN)_6$ solution in aqueous acetate buffer. Fig. 4 depicts the color maps of a deposit prepared from the ethanolic extract of *Ficus obscura* leaves. In these experiments, a potential E_T was applied to the tip electrode that was sufficiently positive (+0.30 V) to promote the oxidation of $Fe(CN)_6^{4-}$ under diffusion-controlled conditions. When null potential was applied to the substrate (Figure 3a), the particles of the insulating compounds deposited on the base Pt electrode appeared as negative feedback features forming more or less irregular aggregates on the electrode surface. Application of a positive potential E_S to the film promoting the oxidation of polyphenolic compounds results, in several regions, in small variations in the film topography (Figure 3b), attributable to the contraction/expansion of the crystals associated to the changes in composition, as already described for the case of flavonoids,[24,47] consistently with the proton-assisted solid state nature of the

involved electrochemical processes. In other regions, however, the application of oxidative potential inputs determines the entire disappearance of the negative feedback, a feature attributable to the presence of compounds whose oxidation yields water-soluble species.

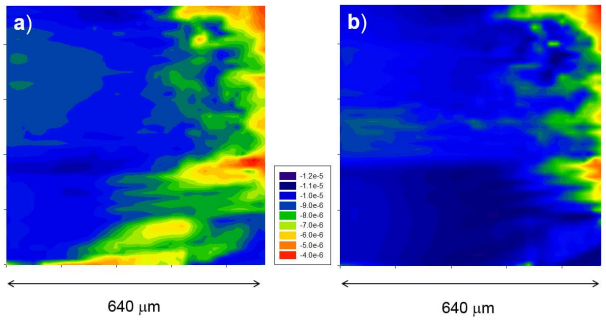

Figure 3. SECM color maps of a microparticulate film from an ethanolic extract of *Ficus obscura* deposited on Pt substrate immersed into 2.0 mM $K_4Fe(CN)_6$ plus 0.25 M HAc/NaAc, pH 4.75. a) E_T = +0.30 V; E_S = 0.00 V; b) E_T = +0.30 V; E_S = +0.60 V.

Repeatability tests were performed using: i) three different leaves harvested the same day under similar conditions; ii) three independently prepared deposits of each one of the studied extracts on the same glassy carbon substrate electrode and iii) three independently prepared deposits of each one of the leaf extracts on different substrate electrodes. In all cases, peak potentials were reproduced with a maximum dispersion of ±10 mV whereas the voltammetric pattern (in terms of current ratios relative to the maximum peak current) remained within a 5-10% of relative standard deviation. Accordingly, the voltammetric records such as in Figure 1 can be viewed as an 'electrochemical fingerprint' characterizing each vegetal species.

3.2. Electrochemical taxonomy

In order to establish the possibility of an electrochemistry-based taxonomy, the voltammetric patterns were correlated with the different taxonomic levels. As can be seen in Figure 1, the leaf extracts of the different species provide clearly different voltammetric patterns in the region of potentials between 0.0 and +1.20 V. Such voltammetric patterns would be representative of the different composition of polyphenolic compounds, thus resulting in highly specific voltammograms. This specificity was maintained using acetone and chloroform extracts. The voltammetric pattern for leaf extracts obtained immediately after harvesting exhibited a certain degree of dispersion which disappeared after few hours. Accordingly, the leaf samples were stored in a desiccator at room temperature (298 K) for 24 h prior to electrochemical experiments. The voltammetric pattern (*vide infra*) remained essentially invariable for at least three-four weeks.

Remarkably, in several orders and families, the studied species displayed a significantly common electrochemical response. This can be seen in Figure 4 for the case of three species of the order Fabales, namely, *Genista tinctoria*, *Quillaja saponaria* and *Dorycnium pentaphyllum* whose leaf extracts using ethanol and acetone were, respectively, similar. In general, the voltammetric response of the extracts varied slowly with time. This can be seen in Figure 5, where the voltammograms of ethanolic extracts of *Myrtus communis* (a, b), and *Oxalis articulata* (c, d) taken 24 h (a, c) and 1 month (b, d) after harvesting are compared.

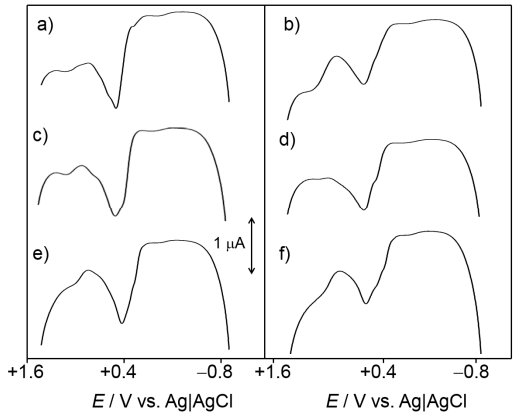

Figure 4. Square wave voltammograms of microparticulate films deposited on GCE of extracts of a,b) *Genista tinctoria*, c,d) *Quillaja saponaria* and e,f) *Dorycnium pentaphyllum* leaves using a,c,e) ethanol and b,d,f) acetone. Electrolyte: 0.25 M aqueous acetate buffer, pH 4.75. Potential scan initiated at −1.05 V in the positive direction; potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz.

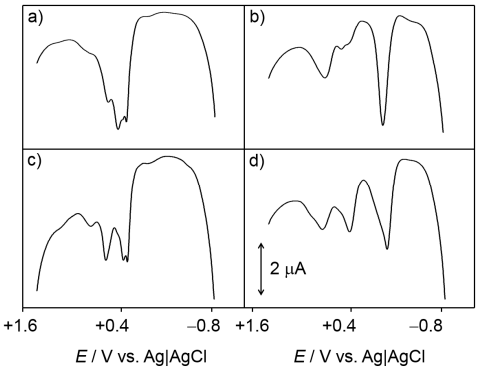

Figure 5. Square wave voltammograms of microparticulate films deposited on GCE of ethanolic extracts of a,b) *Myrtus communis* and c,d) *Oxalis articulata* taken a,c) 24 h and b,d) 1 month after harvesting. Electrolyte: 0.25 M aqueous acetate buffer, pH 4.75. Potential scan initiated at −1.05 V in the positive direction; potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz.

The foregoing set of data suggests that there is a possibility of defining common voltammetric patterns at different taxonomic levels; i.e., that there is a possibility of developing an 'electrochemical taxonomy'. Identification of plant taxonomic groups, speciation studies and monitoring the changes of plant composition during the life of the organism could be made without

need of a detailed analysis of the chemical composition of the leaf extracts.

The above aims require, however, the disposal of pattern recognition criteria. Following literature criteria[25,26,48] normalized voltammograms were constructed as representations of the ratio between the current at a given potential, $I(E)$, and the maximum peak current, I_{max}, vs. the applied potential E. Combining the data for voltammograms recorded from ethanolic and acetone leaf extracts, plots of $(I(E)/I_{max})_{Acetone}$ vs. $(I(E)/I_{max})_{Ethanol}$ provide graphs which should be characteristic of the analyzed vegetal species. The first condition for species characterization purposes: the repeatability of such graphs for replicate experiments on specimens of the same species was accomplished whereas semi-derivative deconvolution was applied to the voltammetric curves in order to increase peak resolution (see Supplementary materials, Figs. S.3-S.5). As expected, the voltammetric pattern varied from one species to another, as illustrated in Figure 6, where plots of $(I(E)/I_{max})_{Acetone}$ vs. $(I(E)/I_{max})_{Ethanol}$, taken at intervals of 4 mV, for films of leaf extracts of *Boehmeria cylindrica*, *Quillaja saponaria*, *Ricinus communis* and *Oxalis articulata*, immersed in acetate buffer are shown. Combining data of voltammograms recorded for films of leaf extracts with different solvents using different electrolytes, one can increase the set of available data to be used for discriminating between different species. Ideally, if each voltammograms provides current data for k potentials, using the extracts for i solvents, each one studied in contact with j electrolytes, a matrix of dimensions $k \times (i\ j)$ can be obtained. Principal Component Analysis using the aforementioned current ratios at different potentials provided a satisfactory grouping of the species pertaining at different families, as depicted in Figure 7. For our purposes, the relevant point to emphasize is that the application to chemometric techniques to this matrix of data would provide discrimination criteria based, exclusively, on electrochemical data *without need of determining the composition of leaf extracts*.

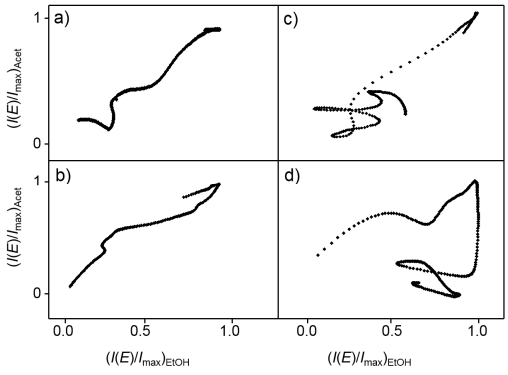

Figure 6. Plots of $(I(E)/I_{max})_{Acetone}$ vs. $(I(E)/I_{max})_{Ethanol}$ for films of leaf extracts of: a) *Boehmeria cylindrica*, b) *Quillaja saponaria*, c) *Ricinus communis* and d) *Oxalis articulata* extracts immersed into 0.25 M aqueous acetate buffer, pH 4.75. Conditions such as in Fig. 2; potentials taken at intervals of 4 mV.

3.3. Correlation with phylogenetic trees

Figure 8 shows a scheme summarizing the presence of voltammetric peaks at potentials within intervals of 50 mV between +250 and +550 mV vs. Ag/AgCl for the species of the Rosales order grouped in their corresponding families. Clearly, such data suggest that family-characteristic patterns exist. In order to establish similarity relationships between the obtained voltammetric responses, hierarchical cluster analysis was performed using the $(I(E)/I_{max})_{Ethanol}$ data from deconvoluted voltammograms of leaf extracts in acetate buffer. Comparison of the obtained dendrogram with the APG III (2009)[1] phylogenetic tree of the Rosid orders revealed clear differences between them (see Supplementary data, Figs. S.6, S.7). However, when $(I(E)/I_{max})_{Solventj}$ data were applied to a lower taxonomic unit, the Rosales order, the obtained dendrogram (see Figure 9) agreed with the phylogenetic tree recently provided by Zhang et al.[35]

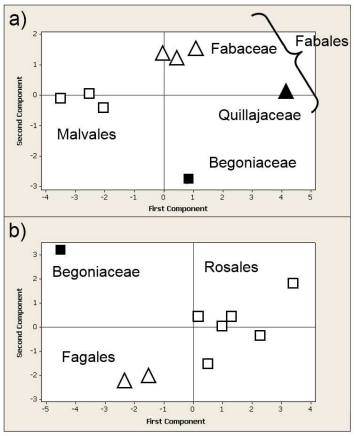

Figure 7. PCA diagrams obtained from square wave voltammograms of a) ethanolic and b) acetone extracts of plants of different orders using current ratios from voltammograms such as in Figures 1, 4 and 5.

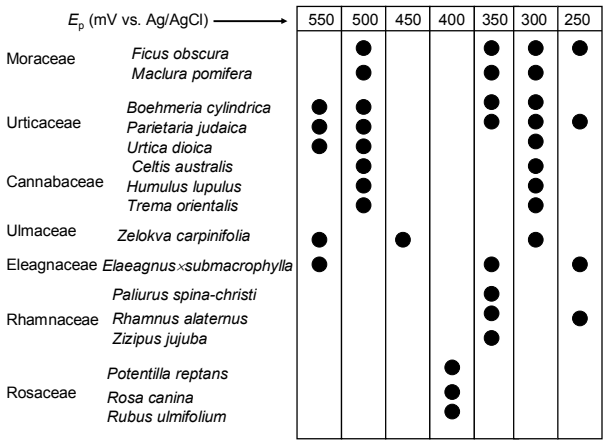

Figure 8. Voltammetric peaks appearing at intervals of potentials of 50 mV between +250 and +550 mV vs. Ag/AgCl in square wave voltammograms of microparticulate films of ethanolic extracts of leafs from species of the Rosales order deposited on glassy carbon electrode immersed into 0.25 M HAc/NaAc aqueous solution at pH 4.75. Conditions such as in Fig. 1.

To rationalize these features, it is pertinent to note that the biosynthesis of many plant chemicals are controlled by genetic factors, but most of them have evolved linked to environment.[49] Plant genetic factors have strong effects but species evolution only could be completely understood within a community and ecosystem context.[50] Accordingly, when phylogenetical trees were compared with the electrochemical response significant divergences may appear and, conceivably, such discrepancies should be larger upon increasing the genetic separation between the compared taxa. Consistently, when comparing the electrochemical signature of species in large phylogenetic groups, such as Rosids, the electrochemical similarity diverges from the genetic one. However, our results suggest that the voltammetric pattern can be correlated satisfactorily with the phylogenetic trees at the taxonomic level of orders.

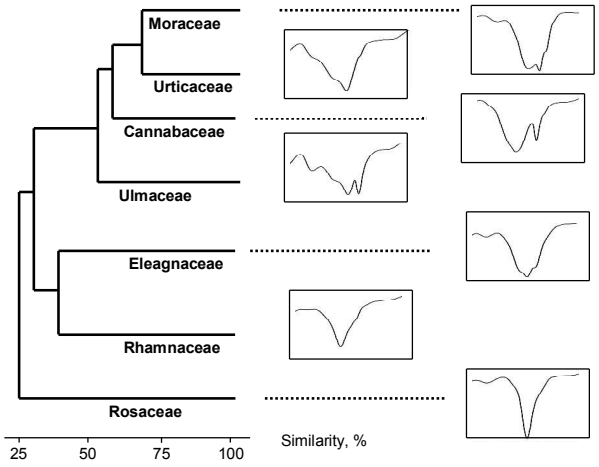

Figure 9. Voltammograms (conditions such as in Fig. 1) of representative species of the main families of the Rosales order superimposed to the dendrogram based on voltammetric data. This dendrogram agrees qualitatively with the phylogenetic tree reported by Zhang et al.[35] using genetic analysis.

A refined analysis of voltammetric data can be performed using peak potential data as illustrated in Figures 8 and 9 and grouping the electrochemical signatures following the appearance of characteristic signals, as already described for discriminating dyes.[51,52] This is illustrated in Figure 10, where the families of the Rosales order are characterized by the presence/absence of different voltammetric signals. In agreement with several phylogenetic studies[35,38,39] Rosaceae, displaying a peak at +400 mV, would be separated from the rest of the order where the peak at +400 mV is absent. The remaining families would be grouped into two distinct clades: one clade centered on Rhamnaceae and relatives, showing a peak at +350 mV, and other comprising Ulmaceae and relatives where the peak at +350 mV is replaced by a signal at +300 mV. In the first of the above two branches, Eleagnaceae would differ from Rhamnaceae by the appearance of an additional peak at +550 mV. Similarly, Ulmaceae would differ from the branch containing Moraceae, Urticaceae and Cannabaceae by the absence/presence of a signal at +500 mV.

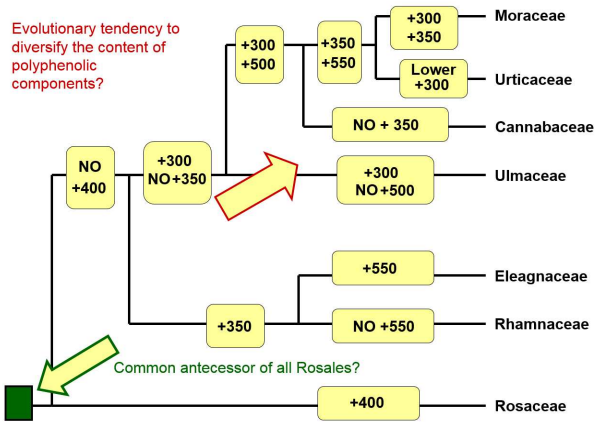

Figure 10. 'Electrochemical tree' of the Rosales order based on the appearance/disappearance of relevant voltammetric signals of ethanolic leaf extracts (conditions such as in Fig. 1) based on the phylogenetic tree reported by Zhang et al.[35]

The above scheme permits to hypothesize several electrochemolomic features of tentative phylogenetic meaning: a) apparently, the ramifications in the tree from the Rosaceae family are accompanied by a complication of the voltammetric response. This would correspond to an evolutionary tendency to diversify the composition of polyphenolic compounds in the Rosales order; b) the sequence of appearance/disappearance of selected voltammetric signals can be interpreted in terms of a temporary sequence of occurrence of genetic changes determining the composition modifications. This would suggest that, in agreement with phylogenetic schemes,[1,35] from a common antecessor of all Rosales, the earlier step was the separation between the Rosaceae and the 'No Rosaceae'. This step would be followed by subsequent genetic modifications which would have their corresponding changes in the 'electrochemical fenotype'; c) combining the above considerations, it would be reasonable to hypothesize that the current Rosaceae would retain the direct heritage of the common antecessor of the order; i.e., that all other families would be 'derived' from the Rosaceae.

It should be emphasized that the 'electrochemical phylogeny' scenario drawn by foregoing set of considerations has to be taken as complementary –obviously, no substitutive– of phylogenetic schemes based on genetic analysis. The 'electrolchemolomic' approach defined here would be of potential interest for assessing phylogenetic linkages and/or tendencies. The accessibility and ease of operation of the involved technique, which even allows for in field analysis using portable equipments and the high representativity of the sampling, because plant extracts are immediately analyzed with no prolonged maceration/extraction, etc. protocols which provide opportunity for aerobic oxidation of much sensitive components, makes the proposed methodology a potentially useful tool for establishing phylogenetic relationships. Additionally, the proposed methodology possesses high intrinsic selectivity, because most of the components of the plant extracts (sugars, cellulose, carotenes, etc.) are electrochemically silent under the described experimental conditions so that only a limited number of compounds (typically, polyphenolic compounds) is electroactive.

4. Conclusions

The voltammetric response of films from ethanol, acetone and chloroform extracts of leaves of different vegetal species immersed into aqueous acetate and phosphate buffers is dominated by anodic signals corresponding to the oxidation of polyphenolic compounds. Such signals mainly correspond to proton-assisted solid state electron transfer processes, as indicated by SECM data, in agreement with abundant evidence from the voltammetry of microparticles technique. The described methodology provides a species-characteristic pattern which can be used to discriminate between different vegetal species, thus defining an 'electrochemical taxonomy' potentially usable for distinguishing between different taxonomic groups complementing existing genetic and chemical analyses. Correlation of electrochemical data with phylogenetic trees for the Rosales order suggests that the electrochemical pattern of leaf extracts can be used to define an 'electrochemobolomic' approximation to phylogenetic data potentially useful for testing phylogenetic relationships and/or tendencies.

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