

Communication

Dechlorophyllation by Electrocoagulation

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Abstract: Electrocoagulation was used for dechlorophyllation of alcoholic extracts from five plants. The results showed that for every plant extract studied, electrocoagulation was more efficient than the classical solvent extraction method in removing plant pigments, while not affecting the important secondary metabolites in those extracts.

Keywords: Dechlorophyllation, electrolytic decolourisation, electrocoagulation

Introduction

In the process of isolation of natural products from plants, the so-called “organic matrix” consisting of such substances as pigments, tannins, carbohydrates, etc. normally must be removed from the crude extract so the desired secondary metabolites may be obtained in a purified form. This problem is more pronounced when the extracted plant part is the leaf, which always contains plant pigments, notably the chlorophylls and the carotenoids, apart from other normally undesirable components, especially the tannins. Conventional methods for removing these substances are solvent extraction and chromatography. However, typically one or more toxic organic solvents must be used in large amounts in both methods, after which these solvents eventually end up as a problematic volatile chemical waste. Also, in chromatography, expensive and usually unrecoverable adsorbents must be used, and even then, the efficiency of pigment removal is usually far from satisfactory. In this report, we present an alternative method of plant pigment removal, in which usage of a large amount of extracting solvents can be dispensed with. The pioneer in this field is Miwa [1], who in 1978 used the process of electrocoagulation to effect dechlorophyllation of an aqueous extract of stevia in order

to obtain the sweet glycosidic components from stevia leaves. However, no further work in which electrocoagulation has been similarly applied has appeared in the literature since then. Instead, electrocoagulation (EC), an electrochemical technique by which a variety of unwanted dissolved particles and suspended matter, both organic and inorganic, can be effectively removed from an aqueous solution by electrolysis, has been mainly used to treat various aqueous organic and inorganic wastes [2-19], or sometimes as a method in clarifying potable water [20-21]. As an extension of the work by Miwa who used only aqueous plant extracts, we report here the dechlorophyllation of alcoholic plant extracts by electrocoagulation. To our knowledge, apart from the work of our group [22-25], there have been no other investigations on electrocoagulation that are performed in solutions containing an organic solvent, the system that is undoubtedly more useful in isolating natural products in general.

Results and Discussion

As shown in Table 1, absorbances at 665-666 nm (corresponding to the green pigments) and at 408-410 nm (corresponding to the yellow pigments) for Solutions 1 (from the EC method) are all lower than the corresponding values for Solutions 2 (from the solvent extraction method). This is also evident visually from Figure 1, thus indicating that dechlorophyllation by EC is more efficient than the conventional solvent fractionation method for all of the plants studied.

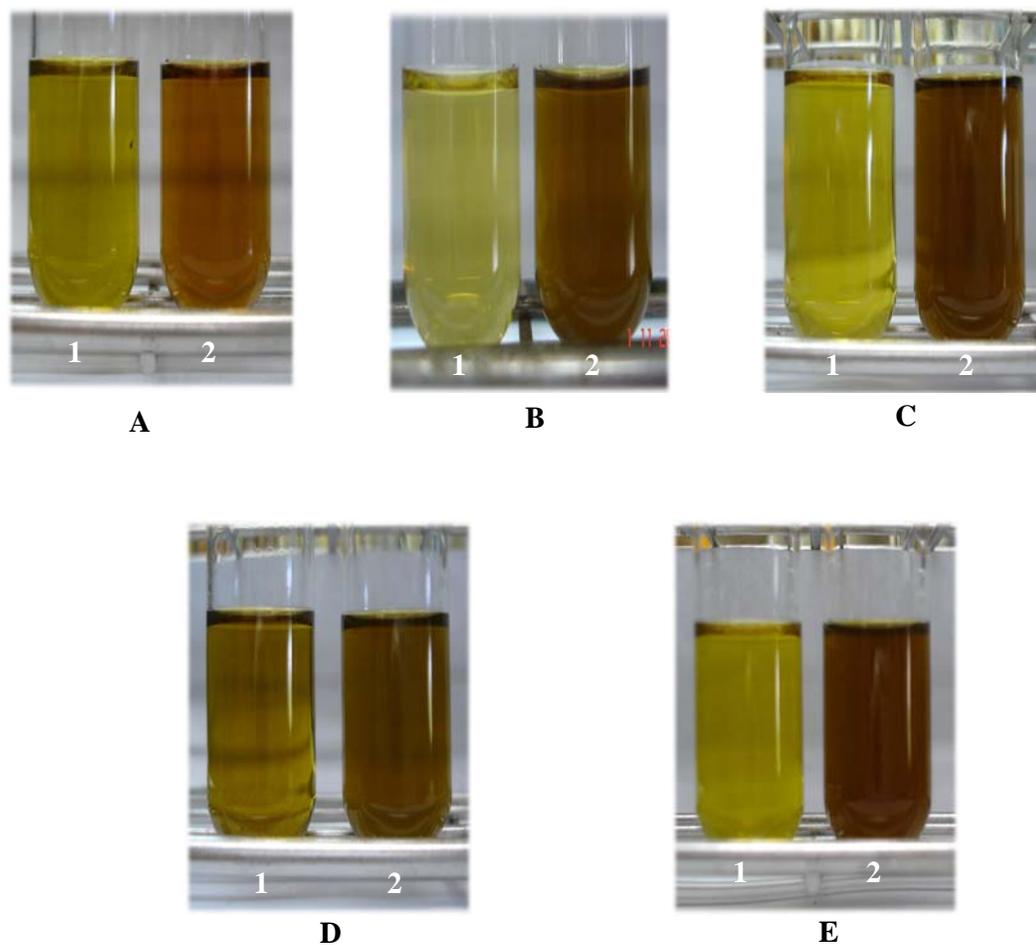
Table 1. Absorbances of plant extracts obtained after dechlorophyllation by EC (Solution 1) and by solvent extraction (Solution 2).

Plant	Absorbance			
	Solution 1		Solution 2	
	665 – 666 nm	408 – 410 nm	665 – 666 nm	408 – 410 nm
<i>Solanum laciniatum</i>	0.35	5.40	1.18	9.03
<i>Andrographis paniculata</i>	0.30	1.85	0.70	5.70
<i>Stevia rebaudiana</i>	0.27	-*	1.50	-*
<i>Centella asiatica</i>	0.65	3.50	1.60	6.50
<i>Cassia siamea</i>	0.30	-*	1.75	-*

* unmeasurable due to interfering peaks.

To ensure that the natural products of interest are not affected by the process of electrocoagulation, we also carried out their isolation from some of the plants. Thus, for stevia leaves, after dechlorophyllation of the alcoholic solution by EC, the sweet glycosidic substances were isolated from the alcoholic extract in the usual manner [26], resulting in a yield of 8-10% for the required glycosides, a normal yield for these compounds obtained by other classical methods in which chemical adsorbents were used for decolourisation.

Figure 1. Plant extracts after dechlorophyllation by (1) electrocoagulation and (2) solvent extraction: A) *Solanum laciniatum*; B) *Andrographis paniculata*; C) *Stevia rebaudiana*; D) *Centella asiatica*; E) *Cassia siamea*.



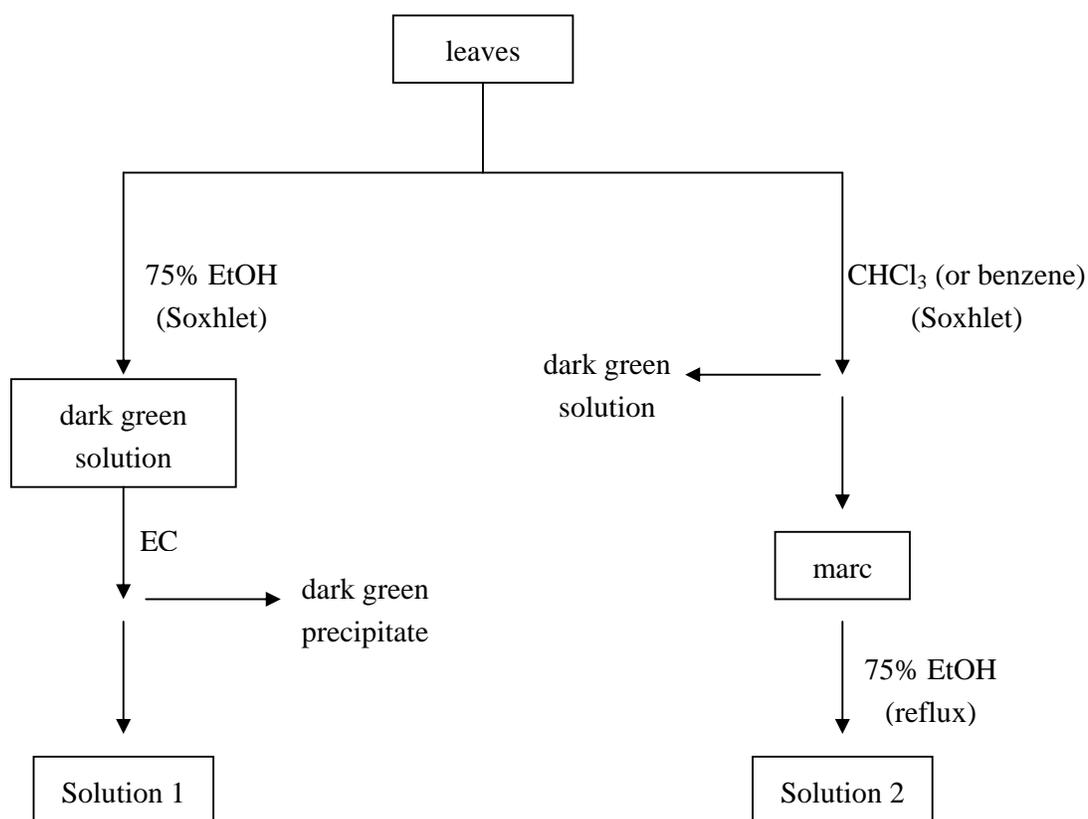
For *S. laciniatum*, solasodine, a steroidal alkaloid used as a starting material for steroid synthesis, was obtained in 1% yield by the EC method. The compound was also isolated from the same leaf samples in only about 0.5% yield by a similar method [27,28] in which chloroform was used conventionally as a dechlorophyllating solvent. For *A. paniculata*, the bioactive lactone content in the crude extract, obtained by simple alcoholic extraction, was about 8% [29,30]. However, when the 75% ethanolic solution, obtained from extracting the leaves from the same source under the same conditions, was subjected to the usual process of electrocoagulation followed by desalting with an ion exchange resin, the resulting crude extract was much more enriched with the lactones (about 38%, as determined by the same method [30]). For *C. asiatica*, our method consisted of extracting the dry leaves with absolute ethanol, diluting the ethanolic solution with water before dechlorophyllating by electrolysis for 2 hours using aluminium electrodes, filtering the resulting electrolysed mixture, evaporating the filtered solution to dryness, and extracting the residue with a little ethanol to afford a crude extract containing 4% (by HPLC) of asiaticoside after evaporation of the ethanol. A classical solvent extraction method [31], in our hands, afforded only 3% (by HPLC) of the same medicinally important glycoside in the crude extract (the asiaticoside content in the dry leaves is about 0.1% [32]).

As for *C. siamea*, we isolated pure D-pinitol, a cyclic polyol, from the dry leaves for the first time by a similar EC method a few years ago [22], although without proper monitoring of the extent of dechlorophyllation as in this report.

Conclusions

Electrocoagulation seems to be a method of choice for dechlorophyllation of some plant extracts, rendering them relatively freer from the interfering plant pigments, and thus leaving the compounds of interest more readily processable. This is in addition to the fact that it is a technique which is more environmentally friendly than the conventional usage of various toxic organic solvents.

Scheme 1.



Experimental

The ground dry leaves of five well known plant species, viz. *Stevia rebaudiana* (stevia), *Cassia siamea*, *Solanum laciniatum*, *Andrographis paniculata*, and *Centella asiatica* (10 g each), which were collected locally, were separately extracted with 75% ethanol (200 mL) in a Soxhlet extractor until colourless. The resulting dark green solution was then subject to electrocoagulation for 2.5 hours.

The conditions for electrocoagulation were as follows: two clean (acetone-washed) aluminium plates (iron plates in case of *S. laciniatum*), each of 3x15 cm dimensions were used as electrodes. These were spaced 1.5 cm apart and dipped 7 cm into the magnetically-stirred solution containing

0.1% (w/v) NaCl as supporting electrolyte. Direct current (0.9 A, 16.9-31.6 V) from a power supply was then passed *via* the two electrodes through the solution, which was placed in a jacketed 250-mL beaker for occasional cooling during electrolysis. After 2.5 hours of electrolysis, the mixture in the beaker was filtered to afford a decolourised solution (Solution 1, 150 mL), which was taken for absorbance measurement to determine the extent of decolourisation at 665-666 nm (corresponding to the presence of the green pigments) and at 408-410 nm (corresponding to the presence of the yellow pigments).

As a comparison, dechlorophyllation by solvent extraction was performed by extracting the same amount of plant material (10 g) with an organic solvent (chloroform or benzene) using a Soxhlet extractor until colourless. After drying 75% ethanol (150 mL) was then added to the marc and the mixture was refluxed for 2 hours, then filtered and the solution obtained (Solution 2, 150 mL) was taken for absorbance measurement as above (See Scheme 1).

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