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First results on the characterisation of the ancient *folium* dye

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The *folium* dye, extracted from *Chrozophora tinctoria* (L.) A. Juss., is cited in several ancient treatises as a pictorial material to obtain blue-purple hues (Dodwell 1961; Brunello 1971; Smith & Hawthorne 1974). Therefore its use in painting art must have been common, in particular with main concern to miniature painting. Nevertheless this dye has been rarely identified on artworks (Guineau 1997) possibly due to the lack of diagnostic information. In addition, at present the comprehension of the chemical nature of the dye is almost entirely missing.

In order to fill this lack, in a recent work an improvement of the spectroscopic information concerning folium has been proposed (Aceto 2015), allowing to evidence its presence on some Western European manuscripts. The same authors started a study in order to characterise the chemical behaviour of folium and to gain information on the structure of the molecules composing it. Preliminary experiments have been carried out in order to verify the reliability of the information emerging from ancient treatises. A relevant feature, always cited in the description of folium, is the behaviour of the dye in relation to pH: Theophilus mentions *folium rubeum*, *folium purpureum* and *folium saphireum*, which should be stable in acid, neutral and basic pH, respectively, but this resulted incorrect as the only difference in colour actually derives by the maturation state of the pericarps of *Chrozophora tinctoria* fruits, which yield a blue hue when fruits are unripe (*folium saphireum*) and purple colour with ripe fruits (*folium purpureum*). Moreover, a red solution can be obtained upon addition of Fe^{3+} ions, suggesting that Theophilus' *folium rubeum* could rather be a Fe-folium complex. Another surprising feature of folium was its behaviour in water and organic solvents. Folium is both *hydrophilic*, being very soluble in water, and *hydrophobic*, as the coloured fraction of an aqueous extract can be completely adsorbed on a reversed phase resin. Most probably its molecules have an *amphiphilic* nature, containing both polar and nonpolar functional groups.

Besides these experiments, instrumental analysis was performed with different techniques (HPLC-MS, SERS-Raman, MALDI-ToF-MS, NMR) in order to define the structure of the dye. For simplifying the matrix, a first purification step on a C18 resin was carried out allowing the elimination of uncoloured hydrophilic compounds and the separation of some coloured fractions, among which a yellow and an orange fraction, most probably rich in flavonoids, and some fractions with different purple tones. Hydrolysis with a pectolytic enzyme was then attempted, in order to remove the hydrophilic part of the molecule which is made of oligosaccharides according to NMR analysis. Works are in progress in order to hypothesise chemical structures of the molecules present in the different fractions.

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