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EXTRACTION AND CHROMATOGRAPHIC SEPARATION OF ANTHOCYANIN IN SORREL

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ABSTRACT: Sorrel is important to the culture of the Virgin Islands for its use in making a healthy red holiday beverage. Fourteen sorrel cultivars obtained from the USDA Germplasm Repository and locally in the Caribbean have been evaluated at the University of the Virgin Islands. The calyx of sorrel varies in the intensity of redness between cultivars. The purpose of this research was to determine the concentration of the red anthocyanin pigment in the calyxes from fourteen sorrel cultivars and use paper chromatography to resolve the pigments. Sorrel calyxes were ground 1/1 (w/v) in either ethanol or water. Following centrifugation, the solute was read in a spectrophotometer at 535 nm. Ethanol was found to be better for extracting the anthocyanin pigment. Paper chromatography, utilizing polar and nonpolar solvents, was used to separate the red anthocyanin pigmented compounds. Ethanol was the most efficient solvent for both extracting the red anthocyanin pigment and resolving the compound with paper chromatography. This research was funded through the VI Dept. of Agriculture Specialty Crops Block Grant and USDA-NIFA- Resident Instruction in Insular Areas (Grant #2008-34816-20016).

Keywords: *Hibiscus sabdariffa*, Roselle, flavonoids

INTRODUCTION

Sorrel is grown for its fleshy tart calyxes used primarily during the holiday season to make a healthy drink that is better than cranberry juice (Appell, 2003; George and Morris, 1984). Multiple varieties of sorrel exist with a range of color from white to dark crimson (Fig. 1). Anthocyanin is the primary pigment that is responsible for the red color and it has antioxidant properties (Marco et al., 2005). The purpose of this research was to quantitate the level of anthocyanin from two extraction solvents and use paper chromatography to separate the pigmented compounds.

MATERIALS AND METHODS

Sorrel calyxes from 14 varieties were harvested at maturity and ten average fruits from each variety were selected. The ten fruit were each divided into two groups of five by variety, cleaned to obtain the calyxes, and the mass was recorded. Each set of calyxes was ground w/v with either 70% ethanol or distilled water in a mortar and pestle. The solution was collected in a 50 ml conical tube and centrifuged 15 minutes at 12,000xg. The supernatant was collected and transferred to a 15 ml conical tube and centrifuged 15 min at 12,000xg. Following the second centrifugation, 1 ml of solute was dispensed into 1 cm cuvette and read in a spectrophotometer at 535 nm. The spectrophotometer was zeroed using either 70% ethanol or water prior to running the samples. Samples registering over 3.0 were diluted 3x or 10x until the reading was within the range.

To determine the pigment composition of the sorrel varieties, 20 uL of the solute was placed 2 cm from the base of 2 x 15cm strip of filter paper. The strips were labeled and placed in a 25 mm test tube containing 3ml 70% ethanol, 95% ethanol, acetone or chloroform as the solvent and ran 12 hours in a fume hood. Sample strips were dried and bands counted.

RESULTS AND DISCUSSION

A spectrophotometric scan from 280-800 nm was run on sorrel solute to determine the point of maximum absorption which was determined to be 535 nm. The solvent found to extract the greatest amount of anthocyanin from the sorrel was 70% ethanol, which was better than water. The day neutral and dark sorrel had the highest concentration of anthocyanins (Fig. 2), concentrations that were at least three times greater than those in the other red varieties. The white, pink, bronze and striped sorrel had low concentrations of anthocyanin (Fig. 2).

During the paper chromatography, both acetone and chloroform had a rapid solvent front. Chloroform, being a nonpolar solvent, didn't move any anthocyanin from the starting sample point indicating that anthocyanin is a polar molecule (Fig. 3). In the acetone, a purplish area remained at and slightly above the sample starting point but ended with a lighter and more diffuse final band (Fig. 3). The purplish area indicates that the acetone may have caused a reaction to precipitate some of the anthocyanin compound so they didn't move to the top with the solvent front. When either ethanol or water was used, all the pigmented anthocyanins migrated up from the sample starting point (Fig. 3). Both water and ethanol are highly polar molecules.

A close examination of the final solvent end point showed four distinct bands formed, indicating that the anthocyanin is made up of multiple forms of the red polar pigment (Fig. 4). The four bands of color may indicate the two main anthocyanins which are delphinidin and cyannidin, possibly with lower levels of their glucosides (Dominiquez-Lopez et al., 2008). Future studies are planned to more accurately quantitate and resolve the compounds using a gas chromatograph and/or high pressure liquid chromatography as has been done by Marco et al. (2005).

CONCLUSION

All 14 varieties of sorrel contained some anthocyanin, even if it was a trace amount as found in the white and light forms. The day neutral and dark varieties had the highest levels of anthocyanin. Ethanol was better at extracting the anthocyanin pigments from calyxes than water. Ethanol and water, being stronger polar molecules, were better solvents than acetone or the nonpolar chloroform for use in paper chromatography to separate the anthocyanin pigments.

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Marco, P.H., M.A.B Levi, I.S.Scarmino, R.J. Poppi, and M.G. Trevisan. (2005). Exploratory analysis of simultaneous degradation of anthocyanins in the calyxes of flowers of *Hibiscus sabdariffa* species by PARAFAC model. Anal. Sci. 21:1523-1527.

Figure 1. Multiple varieties of sorrel indicating variable pigmentation levels.

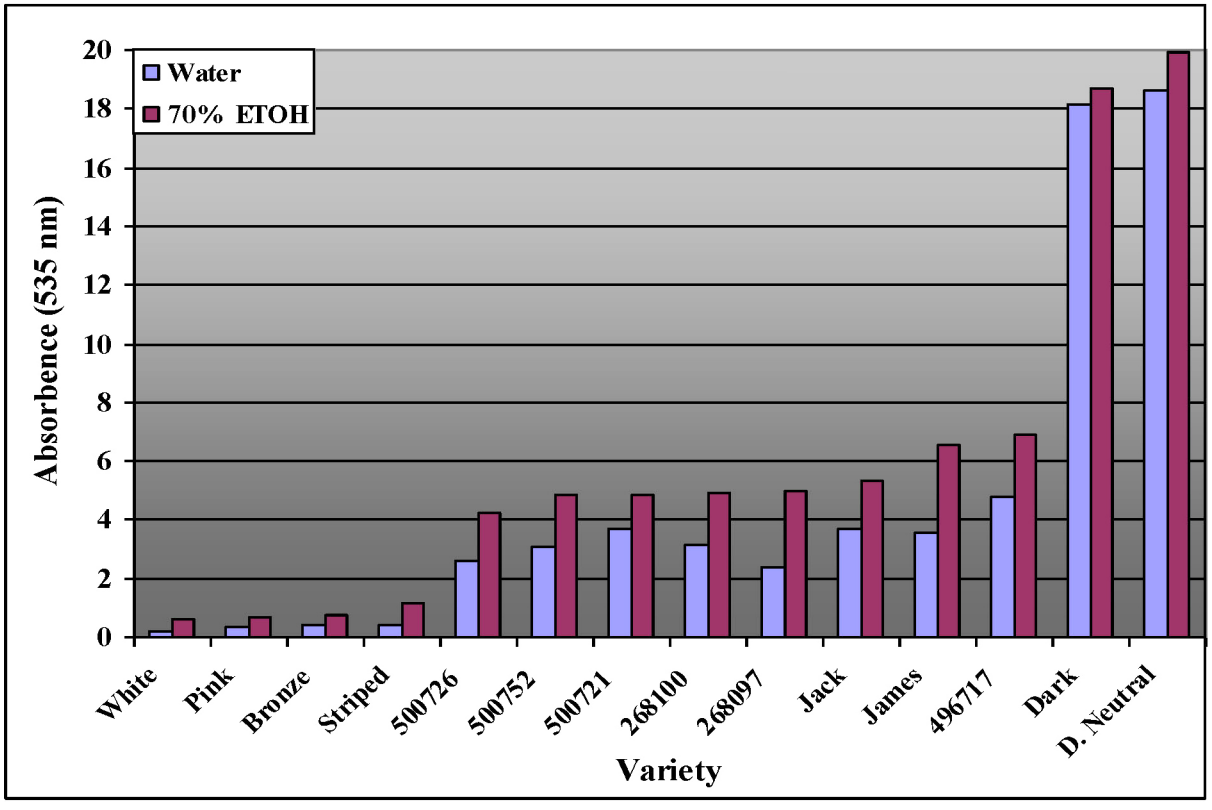
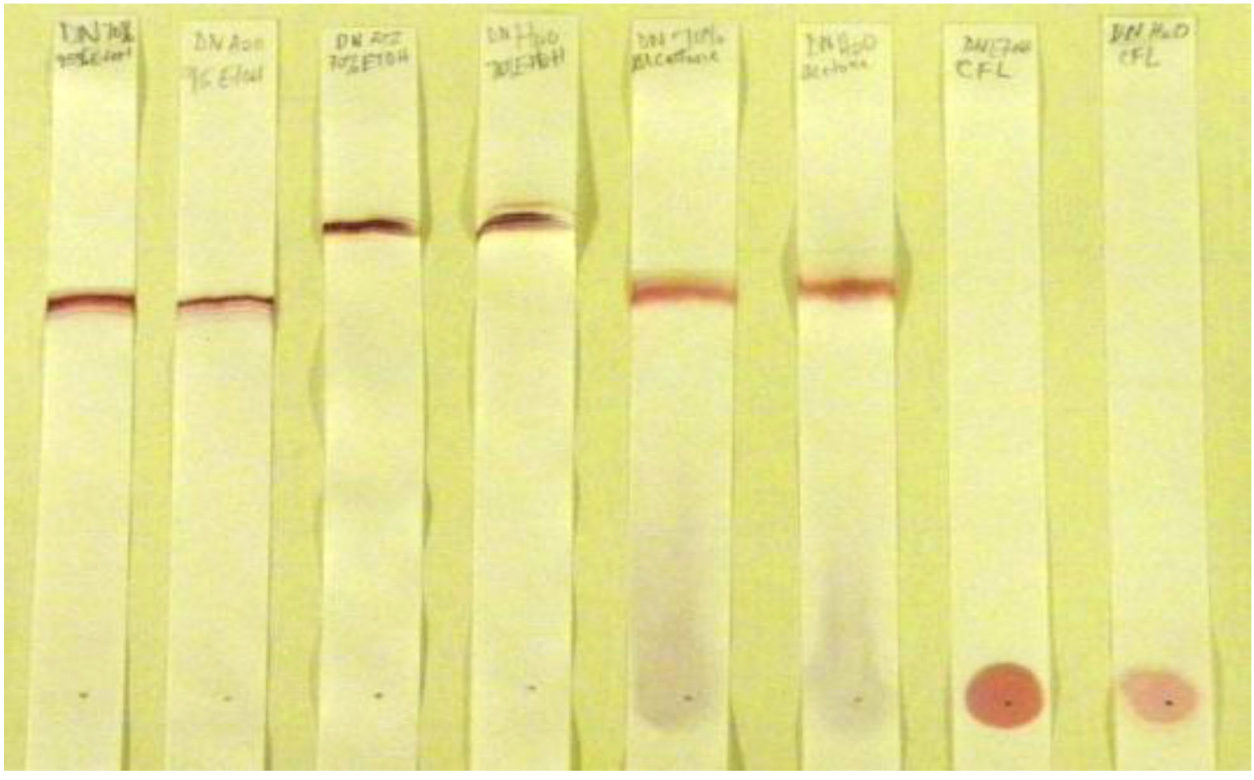


Figure 2. Anthocyanin absorbance level at 535 nm, corrected for dilution, of 14 sorrel varieties extracted with either water or 70% ethanol.



70% ETOH Water 70% ETOH Water 70% ETOH Water 70% ETOH 95% ETOH
95% ETOH 70% ETOH Acetone Chloroform

Figure 3. Extracted pigments resolved on paper chromatography with 95% ethanol, 70% ethanol, acetone or chloroform after 12 hours.

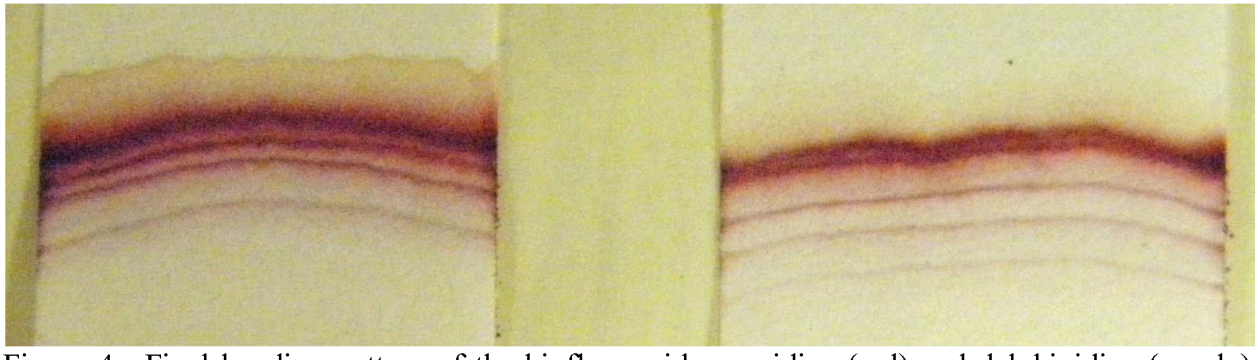


Figure 4. Final banding pattern of the bioflavonoids cyanidins (red) and delphinidins (purple) extracted in 70% ethanol (left) and water (right) resolved in 95% ethanol.