Extraction and Characterization of Water-soluble Pigments from *Saintpaulia* Flowers: Part 1

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Saintpaulia, which is well known as the 'African violet' is such a popular commercial plant in part because of the great variety of flower colors and shapes. These plants mature quickly and flower easily under artificial light. Those characteristics also make the *Saintpaulia* an excellent model organism for researching plant pigments and the enzymes that produce them. The various purple and pink colors of the African violet come from the water-soluble pigments stored in the large central vacuoles of the epidermal cells in the flowers. These pigments are different types of anthocyanins, many of which have been chemically and structurally well characterized. However, on some flowers, the yellow and ivory pigments, which are proposed to be water-soluble flavonoids, have not been well studied yet. Knowing the chemical structure of this molecule would advance our understanding, and identifying which cultivars produce it in abundance will make it easier for people to hybridize many new attractive flowers.

We are in the process of determining the chemical structures of the yellow and ivory pigments that are unusual, and therefore highly sought after, in African violet cultivars. This article (the first in a series) describes how we isolated the water-soluble yellow pigment, and which cultivar has provided the highest yield so far. The result is surprising – it turns out that it is not possible to tell which flowers contain the most yellow pigment just by looking at them. Instead, it is necessary to extract and separate the pigments.

In order to isolate the pigment of interest, we first perform an organic solvent extraction in order to remove the non-water soluble compounds, such as chlorophyll, proteins, and cell membrane components. We do this by first grinding up the flowers and shaking the resulting paste with two solvents that do not mix (think of the fat layer that rises to the top of the water when making gravy), in this case chloroform and acetone. The chlorophyll, proteins, and cell membrane components dissolve in the chloroform layer, while the anthocyanins and our yellow pigment dissolve in the acetone layer, which we need to keep for further experiments. We use a special piece of glassware called a separatory funnel to carefully put the two layers in separate containers **(Figure 1)**.

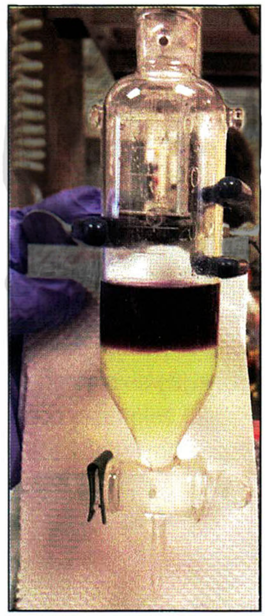

Figure 1: Separatory funnel during chloroform / acetone extraction.

Now we still have the problem that the anthocyanins and our yellow pigment are all mixed up in the acetone layer. We separate them by using a chromatography column, which is packed with silica gel. Silica gel is used in little packets to absorb water in products that need to remain dry – here we use its absorbent properties to separate our compounds. If you have ever seen multiple colors separate from ink on a wet piece of paper, this is the same process. Different pigments stick to the silica gel more or less depending on their chemical structures when we flow the acetone mixture down the column, allowing us to separate them **(Figure 2)**. The purified pigments are shown in **Figure 3**.

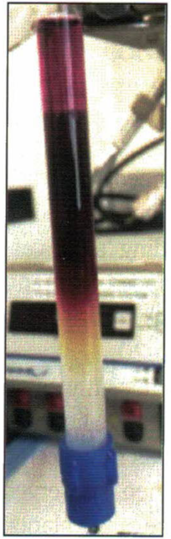

Figure 2: Chromatography column showing the separation of purple and yellow pigments

Figure 3: Purified fractions from the silica gel column.

We tried separating the pigments from many African violet cultivars, and found several anthocyanins as well as varying amounts of the yellow compound. However, we were very surprised by the cultivar that produced the most yellow pigment so far: 'Storm's Eye' (Sorano), shown in **Figure 4**. Based on just looking at the flowers, we would not have expected to find any at all - they look purple to our eyes! This may be interesting to hybridizers who are trying to produce brighter-colored yellow African violets – starting with cultivars like this and breeding for reduced purple color might be easier than starting with less colorful yellow ones and breeding for more color. We hope that someone will try it soon and tell us what happens.

Figure 4: Storm's Eye flowers, which unexpectedly contain a good yield of yellow pigment.