Anthocyanin and Potential Therapeutic Traits in *Clitoria, Desmodium, Corchorus, Catharanthus* and *Hibiscus* Species

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**Abstract**

The USDA, ARS, Plant Genetic Resources Conservation Unit curates several important nutraceutical and medicinal plant species. Anthocyanins are responsible for flower, leaf and seed coat color in plants, and are antioxidants as well. However, little is known about anthocyanin content in *Clitoria ternatea, Desmodium adscendens, Corchorus olitorius, Catharanthus roseus, Hibiscus sabdariffa* and *Acer saccharum*. This study was conducted to identify anthocyanin indexes and additional health enhancing components in these species. An anthocyanin meter with an LED diode of 520 nm was used to measure the anthocyanin index of leaves and flowers from plants growing in the field during July, September and October 2006. We found anthocyanin indexes ranging from 4.1 to 52.4 for leaves and 0.8 to 26.8 for flowers in all five species. The highest leaf anthocyanin index average of 14.6 was found in *D. adscendens* (P1 316623) followed by sugar maple (*A. saccharum*) with a leaf anthocyanin average index of 13.0. *Catharanthus roseus* (P1 608581) also had the highest flower anthocyanin index average of 17.3. These data were recorded at or prior to 50% maturity. In addition, potential health uses for these species are discussed. Phytochemicals identified include but are not limited to an antimicrobial protein from *C. ternatea*, anthocyanin rich extracts from *H. sabdariffa* for potential use as a chemopreventive agent, and antitumor promoters in *C. olitorius* leaves. These anthocyanins and phytochemicals have potential value in the nutraceutical and medical industries. These variable anthocyanin indexes among these species will assist breeders and other scientists with valuable germplasm for development of anthocyanin enriched cultivars.

**INTRODUCTION**

Several genera of economic importance to the nutraceutical as well as the medicinal plant markets are conserved and curated at the USDA, ARS, Plant Genetic Resources Conservation Unit (PGRCU), Griffin, GA. Anthocyanins are responsible for flower, plant, coat color, and recently known to be an antioxidant as well as free radical scavenging (Polya, 2003). Not only have several new anthocyanins been reported in *C. ternatea* (Terahara et al., 1998) but an antimicrobial and insecticidal protein known as finbtin has been discovered as well (Kelemu et al., 2004). The objectives of this research were to compare anthocyanin indexes from these field grown species and to report some additional phytochemicals found in *C. ternatea, D. adscendens, C. olitorius, C. roseus* and *H. sabdariffa* for potential use as a nutraceutical or medicinal plant.

**MATERIALS AND METHODS**

Seed from each of eight legumes including five *C. ternatea*, three *D. adscendens*, as well as one *C. olitorius*, one *C. roseus* and one *H. sabdariffa* accessions were planted in Pro-Mix HP potting soil during mid to late-March inside a greenhouse. After 45–50 days, seedlings from all species were transplanted to field plots of a clayey, kaolinitic, thermic typic kanhapludults soil series at Griffin, GA, in a completely random design. Each plot consisted of 1 row at 6 m x 3 m with 3 m between rows. Data were analyzed using analysis of variance (P<0.001) (SAS Institute, Cary, NC). Mean separations were
conducted using Duncan’s multiple range test (P<0.05).

An Opti Sciences CCM-200 chlorophyll content meter was converted to an experimental hand held anthocyanin meter. The manufacturer replaced the 655 nm light emitting diode (LED) of the CCM with a 520 nm LED in order to measure absorbance near the wavelength at which free anthocyanin aglycones in beans, cyanidin and pelargonidin monoglucosides absorb (Macz-Pop et al., 2004).

Prior to and near 50% seed maturity, anthocyanin indexes were recorded from each of three leaves using this modified anthocyanin meter on four different dates (18 July, 25 September, and 11 or 24 October) from each accession of C. ternatea, D. adscendens, C. olitorius, C. roseus, H. sabdariffa and sugar maple growing in the field. Anthocyanin index data recorded on 11 and 24 October were combined because some accessions produced unsuitable leaves due to senescence. Anthocyanin indexes from sugar maple (Acer saccharum) leaves were included because the similar anthocyanin, cyanidin can be detected with the 520 nm LED (van den Berg and Perkins, 2005). For the first time, additional species including C. olitorius, C. roseus and H. sabdariffa were analyzed for their anthocyanin indexes also using this 520 nm LED.

Leaf tissue from C. ternatea (PI 226265), D. adscendens (PI 237955 and 316623), Hibiscus sabdariffa (PI 291126) and C. olitorius (PI 404028) were prepared for HPLC analysis for the flavonoids myricetin, daidzein, quercetin, genistein and kaempferol following a similar procedure by Wang and Morris (2007).

RESULTS AND DISCUSSION

Anthocyanins

Leaf anthocyanin indexes ranged from 5.5 to 13.9 on 18 July 2006 (Table 1). The species C. olitorius leaves produced the highest but not significantly average anthocyanin index at 12.4 followed by H. sabdariffa leaves (11.6), sugar maple (11.0) and C. ternatea PI 209591 (10.0) (Fig. 1). During 25 September 2006, sugar maple leaves produced a significantly higher anthocyanin index (22.2) than C. roseus. Interestingly as the season progressed on 11 October 2006, PI 316623 of D. adscendens produced a significantly higher leaf anthocyanin index (19.6) than all other species. During 24 October 2006, both PI 316623 and PI 237955 of D. adscendens produced significantly higher leaf anthocyanin indexes (17.4 and 16.9, respectively) than all other species. The three D. adscendens accessions increased by an average of 60% during October in leaf anthocyanin index. Corchorus olitorius produced greater than 100% more leaf anthocyanin indexes in July and September than during October. Anthocyanin indexes were generally stable during July and September with the exception of sugar maple which dramatically increased in leaf anthocyanin index by 100% in September.

For all months combined, anthocyanin flower and leaf indexes ranged from 1.0 to 17.3. The accession PI 608581 (C. roseus) produced the significantly highest flower (dark salmon) anthocyanin index (17.3) followed by D. adscendens (PI 316623) leaves (14.6), dark blue flower of C. ternatea PI 283232 (13.5), sugar maple leaves (13.0), D. adscendens PI 237955 (12.7) and H. sabdariffa PI 291126 (11.1). The white flower of C. ternatea PI 209591 produced the significantly lowest anthocyanin index of 1.0 while the blue flower produced by C. ternatea (PI 226265) and leaves from C. roseus yielded significantly lower anthocyanin indexes of 3.1 and 5.9, respectively, as well (Fig. 2).

Corchorus olitorius produced the most quercetin (578 ng/µl) followed by H. sabdariffa (280.8 ng/µl) and C. ternatea (161.6 ng/µl). Clitorai ternatea leaves produced the most kaempferol (658.6 ng/µl) followed by H. sabdariffa (183.4 ng/µl). Negligible amounts of quercetin and kaempferol were found in both D. adscendens accessions (Fig. 3). Interestingly, quercetin and kaempferol are isoflavones with similar biochemical pathways as the anthocyanin, cyanidin and pelargonidin.

Potential Nutraceuticals and Medicinals

The African traditional medicinal plant, D. adscendens exerted analgesic effects in
mice (N'gouemo et al., 1996) and is used for asthma in Ghana, Africa plus ovarian inflammation in Mato Grosso, Brazil (Barreto, 2002). A soluble dietary fiber from *C. olitorius* suppressed blood glucose elevation in rats and humans (Innami et al., 2005). *Corchorus olitorius* has been found to inhibit the malaria parasite (*Plasmodium falciparum*) above 96% (Sathiyamoorthy et al., 1999).

The common ornamental plant, periwinkle (*Catharanthus roseus*) has been shown to be effective against leukemia, skin cancer, lymph cancer, breast cancer, and Hodgkin’s disease (Kintzios et al., 2004). *Hibiscus sabdariffa* has anticancer properties (Hou et al., 2005) as well as inhibition of LDL oxidation plus potential service as a chemopreventive agent (Chang et al., 2006). See Table 2 for additional potential nutraceuticals and medicinals from these species.

**CONCLUSIONS**

Even though some significant differences for leaf and flower anthocyanin indexes occur among these species tested, several species decrease in anthocyanin index while others increase as the season progresses from 18 July till 24 October. Anthocyanin indexes remained stable for the majority of the species, however sugar maple increased dramatically on 25 September. During plant senescence on 24 October, anthocyanin indexes for both *C. olitorius* and sugar maple dropped while *H. sabdariffa* and *D. adscendens* increased. Anthocyanins provide chemical defenses against pests and may help protect plants during excessively cold days. Anthocyanins also increase in plants as the season progresses and can be observed in some species. For example, *D. adscendens* and *H. sabdariffa* produced green leaves containing large areas of purple leaf color with progressively darker purple leaf areas as the season extended into October.

Dark flower colors in *C. roseus* and *C. ternatea* produced the greatest anthocyanin indexes overall during the entire season while green with purple tinges in leaves of *D. adscendens* and *H. sabdariffa* produced high anthocyanin indexes as well. Anthocyanin indexes were very low from those species producing light colors such as white or blue flowers and green leaves. Further testing should be considered for environmental effects on anthocyanin indexes. However, in this first report for anthocyanin indexes from these species, we suggest that several accessions from within these species need testing to verify potential use in breeding programs with eventual cultivar development for enriched anthocyanin indexes. This hand held anthocyanin meter can be successfully used to estimate anthocyanin indexes from these species tested.

Nutraceutical and phytopharmaceutical species do exist in the USDA, ARS, National Plant Germplasm System at the Plant Genetic Resources Conservation Unit, Griffin, GA. Not only do some of these species provide current nutraceutical uses but many others have potential to be used in this type of market as well as for medicinal uses.

**Literature Cited**


Chang, Y.C., Huang, K.X., Huang, A.C., Ho, Y.C. and Wang, C.J. 2006. *Hibiscus anthocyanins*-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages


Table 1. Range of leaf and flower anthocyanin indexes from *Clitoria ternatea*, *Desmodium adscendens*, *Corchorus olitorius*, *Catharanthus roseus*, and *Hibiscus sabdariffa* accessions growing in the field at Griffin, GA during July, September and October 2006.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Anthocyanin Index Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>July 18</td>
</tr>
<tr>
<td><strong>Leaves</strong></td>
<td></td>
</tr>
<tr>
<td><em>Clitoria ternatea</em></td>
<td>5.5–13.4</td>
</tr>
<tr>
<td><em>Desmodium adscendens</em></td>
<td>7.2–12.7</td>
</tr>
<tr>
<td><em>Corchorus olitorius</em></td>
<td>12.0–13.2</td>
</tr>
<tr>
<td><em>Catharanthus roseus</em></td>
<td>7.9–9.0</td>
</tr>
<tr>
<td><em>Hibiscus sabdariffa</em></td>
<td>10.4–13.9</td>
</tr>
<tr>
<td>Sugar maple</td>
<td>8.2–13.3</td>
</tr>
<tr>
<td><strong>Flower (color)</strong></td>
<td></td>
</tr>
<tr>
<td><em>Clitoria ternatea</em> (PI 209591) (white)</td>
<td>1.0–1.2</td>
</tr>
<tr>
<td><em>C. ternatea</em> (PI 283232) (dark blue)</td>
<td>7.1–9.8</td>
</tr>
<tr>
<td><em>C. ternatea</em> (PI 226265) (blue)</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Catharanthus roseus</em> (dark salmon)</td>
<td>19.5–23.9</td>
</tr>
</tbody>
</table>
Table 2. Potentially useful phytochemicals in *Clitoria, Desmodium, Corchorus, Catharanthus* and *Hibiscus* species.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Potential therapeutic phytochemical</th>
<th>Potential use</th>
<th>Plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clitoria ternatea</em></td>
<td>Anthocyanin</td>
<td>Pesticidal (Kelemu et al., 2004)</td>
<td>Flower</td>
</tr>
<tr>
<td></td>
<td>Finotin</td>
<td></td>
<td>Seeds</td>
</tr>
<tr>
<td><em>Desmodium adscendens</em></td>
<td>Ethanolic extract</td>
<td>Analgesic (N’gouemo et al., 1996)</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Corchorus olitorius</em></td>
<td>Phenolic compounds</td>
<td>Antioxidative (Azuma et al., 1999)</td>
<td>Leaves</td>
</tr>
<tr>
<td></td>
<td>Soluble dietary fiber</td>
<td>Suppress blood glucose elevation (Innami et al., 2005)</td>
<td>Leaves</td>
</tr>
<tr>
<td></td>
<td>Phytol</td>
<td>Antitumor promoter (Furumoto et al., 2002)</td>
<td>Leaves</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td>Inhibited malaria parasite &gt; 90% (Sathiyaamoorthy et al., 1999)</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Hibiscus sabdariffa</em></td>
<td>Extract</td>
<td>Antibacterial (Zakaria et al., 2006)</td>
<td>Leaves</td>
</tr>
<tr>
<td></td>
<td>Anthocyanins</td>
<td>Hypertension, pyrexia, liver disorders (Chang-Che et al., 2003)</td>
<td>Flower (dried)</td>
</tr>
<tr>
<td></td>
<td>Phenolic compounds</td>
<td>Inhibit LDL oxidation, apoptosis for chemoprevention (Yun-Ching et al., 2006)</td>
<td>Calix (dried)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anticancer (Hou et al., 2005; Chang et al., 2005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antioxidant, antimutagenic, radical scavenging (Farombi and Fakoya, 2005)</td>
<td>Flower (dried)</td>
</tr>
</tbody>
</table>
Figures

Fig. 1. Mean anthocyanin indexes from six species tested in the field during summer and fall.

Fig. 2. Combined mean leaf and flower anthocyanin indexes from all six species during the entire season (July-October). Those accessions producing similar leaf and flower colors were combined.
Fig. 3. HPLC chromatograms of isoflavones identified in *D. ascendens*, *C. ternatea*, *C. olitorius* and *H. sabdariffa*. 