Effect of Light Intensity on the Pigment Composition and Oxalic Acid Concentrations in Kalamegh (Andrographis paniculata) Leaf

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Keywords: chlorophyll, oxalic acid, photosynthesis

Abstract
Kalamegh (Andrographis paniculata, Acanthaceae), also called the andrographis or “King of Bitters” is native to the Asian subcontinent. The roots and leaves of this herb have been used for centuries by the traditional medical practitioners of India and China to treat numerous ailments ranging from poor digestion and throat infections to hepatitis. A characteristic of the Kalamegh leaf is that it changes its color from green to red and vice versa from time to time, when exposed to varying intensities of light and shade. While Kalamegh has been extensively studied for its phytochemistry and pharmacological composition, safety, efficacy, and mechanisms of action, few studies report the details of the changes in pigment composition of the leaves. The objective of the study was to study the pigment composition and oxalic acid concentrations of the leaves to understand the color change process and its probable effect on the photosynthetic process. Leaves exposed to bright sunlight had up to 35% lower chlorophyll and 30% higher oxalic acid concentrations, indicating that the change in pigment composition and oxalic acid concentrations may be defensive mechanisms to protect the photosynthetic apparatus from bright light.

INTRODUCTION
Andrographis (Andrographis paniculata, Acanthaceae) is native to the Asian subcontinent. The roots and leaves of this herb have been used for centuries by the Ayurvedic practitioners of India to treat numerous ailments ranging from poor digestion to hepatitis. Similarly, in the Chinese medical tradition, the plant has been used to treat a number of health problems including gastrointestinal complaints and throat infections. It is called Bhunimba in Sanskrit, Kalamegh in Hindi, and “king of bitterness” in English and all these names reflect its extreme bitter taste. Due to its wide range of uses and popularity, it is also called the “Indian echinacea.”

Cultivation
Andrographis is cultivated in Asia specifically in India, Sri Lanka, Pakistan, Indonesia, Malaysia, China, Thailand, the East and West Indies, and Mauritius. It is propagated from seeds and thrives well in a wide range of soil types and sunny climates. The seedlings are transplanted at a distance of 2ft (60 cm) between rows and 1ft (30 cm) between plants. Studies report variability in the concentration of the active compound in andrographis (andrographilide) in the different accessions (Sabu et al., 2001), and due to stage of harvest (Patel et al., 1999).

Botany
Andrographis is an erect, annual herbaceous plant, growing to about 1.6-6.2 ft (0.5 to 1 m) in height and branching profusely. The stem is dark green, quadrangular with longitudinal furrows and slightly swollen at the nodes. The leaves are simple, entire, opposite, glabrous, lanceolate and pinnate. The flowers are small, bisexual, white with violet markings, and very irregular in shape borne as axillary racemes or terminal panicles. The sepals are green and the petals bilabiate, white in color with specks of purple. The fruits are capsules, linear and oblong, tapering at both ends with numerous
yellowish brown seeds.

**Phytochemistry**

The bioactive compounds isolated and identified in andrographis include diterpenoids (1) over 24 diterpenoids have been isolated from the leaves of kalamegh. The bitter colorless and crystalline Andrographolide (Fig. 1) is the primary active compound in this plant species. (2) Flavonoids: over 19 flavonoids have been isolated from the leaves of Kalamegh. (3) Other chemicals isolated include steroids, polyphenols, polysaccharides, and lactones. Recently, the various groups of chemical compounds isolated from andrographis and the pharmacology were extensively reviewed (Saxena et al., 1997). The leaves contain the highest amount of andrographolide (2.39%), while the seeds contain the lowest amount of this active principle.

**Traditional Uses**

The whole plant is harvested and dried in shade. The crude drug consists of dried or fresh leaves or the aerial portions of the plant. Sometimes, the whole plant, including the roots, is used for the medicinal preparations. Andrographis is used in Asia to treat gastrointestinal tract and upper respiratory infections, fever, herpes, sore throat, diarrhoea, dysentery, worms jaundice and a variety of other chronic and infectious diseases (Chopra et al., 1956, 1982). It is listed in the Indian Pharmacopoeia and is a major ingredient in numerous Ayurvedic formulas. In Traditional Chinese Medicine (TCM), andrographis is an important herb proposed to have a “cold” property and a “bitter” taste entering the lung, stomach, large intestine, and bladder meridians and channels removing toxins. It is used to cool an excessively heated body, to treat fevers, as well as to get rid of toxins from the body. It is also used in the treatment of infectious diseases, tonsillitis, bronchitis, pneumonia, whooping cough, dysentery, gastric problems, tuberculosis, gall bladder infection, flu and fevers and high blood pressure. In Scandinavian countries, it is commonly used to prevent and treat common colds (Perry and Metzger, 1980). In India Kalamegh is mentioned in ancient medical texts - it was recommended in Charaka Samhita (175 BC) for treating jaundice, in the treatment of colic dysentery and dyspepsia, as a tonic, antispasmodic, antiperistaltic, stomachic and anthelmintic, for fevers, liver disorders, and dysentery, the juice of fresh leaves used to treat colic pain, loss of appetite, irregular stools and diarrhea, stomach complaints and typhoid fever (Sharma, 1983). In China, andrographis is used both as an injection and in tablet form, for dysentery, upper respiratory tract infections, tonsillitis and other infectious diseases (Wagner and Farnsworth, 1990). Andrographis is known as Fah Talai Joan in Thai Folklore, used in the treatment of various diseases of the liver, colic, fevers, and as anthelmintic. In Sweden, the formula of Andrographis is called Kan Jang, it is used as a preventive formulation against common cold. In Malaysia, andrographis is known as Hempedu Bumi for Malaysians and for the Javanese it is commonly known as Sambiloto or Sambiroto. The stems and leaves are used to reduce blood sugar levels and hypertension.

**Current Uses and Health Value**

Andrographis has been extensively studied for pharmacological composition, safety, efficacy, and mechanisms of action. There is abundant scientific research on this plant species that confirms the broad range of pharmacological effects proposed in ancient Asian Medical systems. The medicinal properties demonstrated of the Andrographis extracts in controlled studies and human clinical trials include as an analgesic (Madav et al., 1995), anti-inflammatory (Madav et al., 1996), antipyretic (Vedavathy and Rao, 1991; Wang et al., 1999), antithrombotic (Zhao and Fang, 1991), cardioprotective (Liang et al., 1996), antiulcerogenic (Madav et al., 1995), anti diarrheal (Gupta et al., 1990), choleric (Shukla et al., 1992), preventing of common colds and as expectorant (Caceres et al., 1997; Hancke et al., 1995), hepatoprotective (Chander et al., 1995; Pramnothet al., 1994; Ram, 2001; Saraswat et al., 1995; Trivedi and Rawal, 2001), enhancing the immune system (Puri et al., 1993), enhancing sleeping quality
(Mandal et al., 2001), hypoglycemic and antidiabetic (Zhang and Tan, 2000a; Zhang and Tan, 2000b), antiallergic (Gupta et al., 1998; Madav et al., 1998), hypotensive (Zhang and Tan, 1996), antimalarial (Misra et al., 1992; Najib et al., 1999), in treating HIV patients (Calabrese et al., 2000; Weibo, 1995), protecting against filarial parasitic infections (Zaridah et al., 2000), and as an antifertility promoter (Akbarsha et al., 1990; Zoha et al., 1989). Other studies provide evidence of chemopreventive potential of the andrographis extracts against carcinogenicity (Singh et al., 2001).

Effect of Light Intensity on Chlorophyll and Oxalic Acid Concentrations

Patel et al. (1999) described the differences in the andrographolide and Fe content of andrographis leaves harvested at 15, 30, 45, 60, 75, 90, 105 and 140 d after transplant. However, they do not report the oxalic acid concentrations in the leaves harvested at different stages of harvest. Although the change of color of andrographis leaves under different light intensities has been reported earlier, this property has not been linked to oxalic acid concentrations or studied as a plant defense mechanism. Since the availability of Fe in leaves is influenced by oxalic acid concentrations this study was undertaken to examine the influence of light intensity on the oxalic acid as well as the chlorophyll concentrations, and examine the role of oxalic acid and chlorophyll concentrations in leaves as a plant defense mechanism against excess light.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Twenty-one days old seedlings of Andrographis were transplanted into 500-cm$^3$ square pots in the greenhouse. The plants were fertilized with 100 ppm N-P-K fertilizer mix. At 30 d after transplant half of the plants were moved to an interior part of the greenhouse that only received 50% of sunlight though out the day, compared to the plants that were kept in the bright sunlight. The light intensity in the greenhouse was measured using a Quantum Sensor (Model LI-190SA, LI-COR, Inc., Lincoln, NE). The plants at high light intensity received an average of 585 µmol m$^{-2}$ s$^{-1}$ and the plants kept under partial shade received 345 µmol m$^{-2}$ s$^{-1}$.

Harvest and Data Collection

At 60 days after transplant, plants from the two treatments were harvested and whole plant fresh weight, and dry weight were determined. Leaf discs were extracted with N,N-dimethyl formamide and the chlorophyll concentrations were determined in triplicates for the two treatments. Fully expanded young leaves (3rd, 4th, and 5th nodes from shoot tip) were harvested, dried at 60°C for determining the oxalic acid concentrations. Data were analyzed using paired t-test.

Oxalic Acid Determination

The oxalic acid concentration of the leaf was determined using the procedure as described by Ilarslan et al. (1997). 0.01 g of dry leaf sample was ground with 5 mL de-ionized water. 5 mL EDTA (1M) was added and filtered with Whatman No. 1 filter paper. The oxalate kit purchased from Sigma (Oxalate urinalysis diagnostic kit: procedure No. 591, Sigma, St. Louis, Missouri) was used for the determination of the leaf oxalic acid concentrations: oxalate reagents were warmed to 37°C; test tubes were labeled for blank, control, standard and sample; 1 mL oxalate reagent A (DMAB (3-dimethylamino) benzoic acid + MBTH (3-methyl-2-benzothiazolinone hydrazone), pH=3.1) was added to each tube; 50 µL of sample were added to each sample tube; 50 µL deionized water were added to the blank and control tubes; 50 µL oxalate standard were added to the standard tube; 0.1 mL of oxalate reagent B (oxalate oxidase and peroxide) was added to all tubes and immediately mixed by gentle inversion. All tubes were incubated at 37°C for 5 min. The absorbances of blank, control, standard, and sample were determined at 590 nm in a Thermo Spectronic UV/VIS spectrophotometer (Rochester, NY). Measurements were
taken twice to obtain consistent absorbances. Corrected absorbances were determined by subtracting the blank absorbance from absorbance readings of standard, control and the sample.

RESULTS

Results show that the oxalic acid and chlorophyll concentrations of Andrographis plants were influenced by the light intensity to which the plants were exposed (Table 1). The plants grown under partial shade had 23% lower oxalic acid concentrations and 53% higher chlorophyll concentrations compared to the leaves of plants grown under higher light intensity. The shoot fresh and dry weight were not altered by the light intensities under which the plants were grown.

CONCLUSIONS

The results of the study indicate that the phytochemistry of andrographis leaves can be altered by growing it under different light intensities. In folk and traditional medicine Andrographis is well known for its high Fe content and taken as a remedy for Fe deficiency. Since the availability of Fe in green leaves is dependent on the oxalic acid concentrations, this study provides valuable information to reduce the oxalic acid concentrations to enhance the availability of Fe in the andrographis leaves. Previous studies have demonstrated that stage of growth at harvest as well as variety can have an effect on andrographilide and Fe content of leaves and whole plants (Sabu et al., 2001; Patel et al., 1999). Patel et al. (1999) studied the dry matter yield, andrographilide and Fe concentrations in different parts of the andrographis plant when harvested at different days after transplant and reported that although the leaf dry mass yield was the highest when harvested at 60 days after transplant, the Fe content was the lowest. The results of this study show that in leaves harvested 60 d after transplant, the oxalic acid concentrations were 23% suggesting possibly higher availability of Fe for uptake. Thus environmental conditions can be altered during crop growth to optimize the oxalic acid concentrations, without decreasing the dry matter yield to enhance the availability of Fe in andrographis, when taken to treat anemia. More detailed studies are planned to study the complete pigment profile under different light intensities.

Literature Cited


Tables

Table 1. Plant growth characteristics, chlorophyll, and oxalic acid concentrations in the leaves of Andrographis plants exposed to bright sunlight and partial shade.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxalic Acid (mmols/g DW)</th>
<th>Shoot Fresh Weight (g)</th>
<th>Shoot Dry Weight (g)</th>
<th>Chlorophyll (mg.g⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sunlight</td>
<td>15.0</td>
<td>58.7</td>
<td>6.2</td>
<td>225</td>
</tr>
<tr>
<td>Partial shade</td>
<td>11.5</td>
<td>54.8</td>
<td>5.6</td>
<td>345</td>
</tr>
<tr>
<td>P value</td>
<td>≤ 0.001</td>
<td>NS</td>
<td>NS</td>
<td>≤ 0.001</td>
</tr>
</tbody>
</table>

Figures

Fig. 1. Andrographolide.