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Short Communication

SHADE CONDITIONS PREVENTS ANTHRAQUINONE PRODUCTION IN LEAVES OF *VERNONIA AMYGDALINA* PLANTS

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ABSTRACT
Anthraquinones are a class of natural products with potential therapeutic value, such as antimicrobial, insecticidal, antitumor, anticongestive, hypotensive and sedative properties. Anthraquinones are produced and accumulated in both growing plants and cell suspension cultures. This report is on the effect of open field and open shade growth conditions on the production of anthraquinone and other phytochemicals in *Vernonia amygdalina*. Stalks of the plant species were grown from stem cuttings and the phytochemical contents were analysed between 42 to 70 days of treatment. The result of this study shows that reduction in illumination prevents anthraquinone production but enhances the production and accumulation of flavonoids in an intact higher plant such as *V. amygdalina*. Growers seeking to cultivate *V. amygdalina* for use as herbal laxative should cultivate the plant under open field while those seeking to avoid the laxative effect of the plant whilst keeping the antioxidant and antimicrobial properties should cultivate the plant under open shade conditions like that used for the study.

KEY WORDS: anthraquinone, *Vernonia amygdalina*, open shade, open field, flavonoids.

INTRODUCTION

Anthraquinones are a class of natural products encompassing several hundreds of compounds, differing in the nature and positions of substituent groups (Schripsema *et al*., 1999). Many anthraquinones have potential therapeutic value, such as antimicrobial, insecticidal, antitumor, anticongestive, hypotensive and sedative properties (Abdulllah *et al*., 1998, Komaraiah *et al*., 2005 and refs therein). Anthraquinones are produced and accumulated in both growing plants and cell suspension cultures. Anthraquinones are common constituents of Rubiaceae, found mainly in roots, leaves, fruits and cell suspension cultures of species belonging to the Rubioideae subfamily (Young *et al*. 1996; Oliveira *et al*., 2007). Aside from Rubiaceae species, cell cultures of plants such as *Morinda citrifolia* L., the Noni fruit (Stalman *et al*., 2003), *Cinchona* 'Robusta' (Han *et al*., 2002), *Photorhabdus luminescens* (Brachmann *et al.*, 2007), *Galium mollugo* L. (Wilson and Balague, 1985) are capable of accumulating substantial amounts of anthraquinones.

Cimanga *et al.* (2004) reported the presence of anthraquinone in whole plants of *Vernonia amygdalina. V. amygdalina* (Del.) is commonly known as Bitter leaf, due to its bitter taste. It belongs to the family Asteraceae, *V. amygdalina* commonly known as bitter-leaf is a shrub that grows up to 3 meters high. *V. amygdalina* is a tropical plant commonly found in Africa, particularly in South Africa, Zimbabwe, and Nigeria. The major constituent is vernonioside, also called vernomin (dycosidal bitter), which is responsible for the bitter taste (Oleszek *et al.,* 1995; Farombi, 2003; Afolayan *et al.,* 2006). The bitterness of its leaves can be abated by boiling or soaking in several changes of water. They are held to be anti-scorbutic and are added to soups or eaten as spinach. Leaves from this plant serve as food vegetable and culinary herb in soup (Argheore *et al*., 1998). *V. amygdalina* is used in local medicine as antiparasitic, antipyretic, laxative (anticongestive) and tonic. In Nigerian herbal homes, extracts of the plant are used as tonic, in the control of tick and treatment of cough, feverish condition, constipation and hypertension (Regassa 2000; Kambizi and Afolayan 2001; Amira and Okubadejo 2007). Strong antioxidant activities have been reported for flavonoids from *V. amygdalina* and, its saponins have been reported to elicit antitumoral activities in leukemia cells (Jisaka *et al*., 1993).

There are conflicting reports on the possibility of the production of anthraquinones in *Vernonia* species. While Cimanga *et al.* (2004) and Ogundare *et al.* (2006) reported the presence of anthraquinone in whole plants of *V. amygdalina* and *V. tenoreana* respectively, Kunle and Egharevba (2009) reported the absence of anthraquinones in whole plants of *Vernonia ambigua*. Laboratory analyses of mixed samples of *V. amygdalina* from open field and open shade conditions showed the presence of anthraquinone in some samples and absence in some. This influenced the study of the effect of open field and open shade growth conditions on the production of anthraquinone and other phytochemicals in *Vernonia amygdalina*.

MATERIALS AND METHODS

Stem cuttings from plants of same variety growing in same location were cultivated in the nursery for two weeks.

Then the cuttings with growing stalks were transplanted to two different sites: The first site was an open field that is under direct sunlight and the second site was a shaded site shielded from direct sunlight by a big *Plumera alba* tree, with many branches. The temperature of the open field site ranged from 29.5 to 33.5 °C while the temperature of the open shade site ranged from 26.0 to 30.5 °C. Light intensity of the open air site ranged from 3142 to 11900 Lux while the light intensity of the open shade ranged from 1546 to 9100 Lux. Watering was done every other day with equal quantity of water. Fresh whole stalks at 42, 56 and 70 days after transplanting were pulverized using mortar and pestle.

Phytochemical screening

The pulverized sample was dried for two weeks. The presence of anthraquinone was determined using standard methods (Sofowora, 2008; Evans 2002). The presences of alkaloids, saponins, flavonoids and reducing sugar were also determined.

RESULTS & DISCUSSION

The temperature of the open field site was 3.5 °C higher than that of the shade, while the light intensity of the shade site was about 23.5 to 50.8 % lower than that of the open air site. Results of phytochemical screening were same irrespective of the age of the stalk. The phytochemical screening of whole plant of *V. amygdalina* (Table 1) shows the presence in those grown under open field but absence of anthraquinones in plants grown under open shade. Alkaloids, saponins and flavonoids were however present in plants grown under both open field and open shade sites. Plants grown under open field had higher flavonoids than those under open shade.

TABLE 1: Phytochemical constituents of leaves of *Vernonia amygdalina* from 42 to 70 days after transplanting

	Growth conditions	
Phytochemical constituents	Open field	Open shade
Anthraquinones	+	-
Alkaloids	+	+
Saponins	+	+
Flavonoids	++	+

++ = Present at higher concentration; + = Present ; - = Absent

van den Berg *et al.* (1988) have reported that light has an effect on anthraquinone production. Their work on suspension cultures of *Rhamnus purshiana* showed that the accumulation of anthracene derivatives (i.e. 1,8-dihydroxyanthraquinones, anthrones and/or dianthrones was increased by a daily photoperiod of 12 hr while the formation of anthra-derivatives was strongly suppressed when the cultures were continuously illuminated. The result of this study suggests that reduction in light intensity of between 23.5 to 50.8% prevents anthraquinone production in intact higher plant such as *V. amygdalina*.

The results also show that while open shade conditions inhibit anthraquinone production, they enhance the production and accumulation of flavonoids in *V. amygdalina*. In human health, flavonoids serve as antioxidants while anthraquinones serve as laxatives (Patel *et al.* 1989). The results of the experiment therefore suggests that growers seeking to cultivate *V. amygdalina* for use as a herbal laxative should cultivate the plant under open field while those seeking to avoid the laxative effect of the plant whilst keeping the antioxidant and antimicrobial properties should cultivate the plant under open shade conditions like that used for the study.

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