Journal of Plant Studies; Vol. 2, No. 2; 2013
ISSN 1927-0461 E-ISSN 1927-047X
Published by Canadian Center of Science and Education

Simulated Drought and Salinity Modulates the Production of Phytochemicals in *Acalypha wilkesiana*

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Received: May 18, 2013 Accepted: June 8, 2013 Online Published: June 10, 2013

doi:10.5539/jps.v2n2p105 URL: http://dx.doi.org/10.5539/jps.v2n2p105

Abstract

In this study, 3-month old *Acalypha wilkesiana* plants grown from stem cuttings were exposed to simulated drought and salinity separately to assess the effects of such abiotic factors on the phytochemical production level in this plant. Preliminary study, based on the qualitative analysis, showed the presence of alkaloids, cardial glycosides, flavonoids, saponins, steroids, and tannins in *A. wilkesiana* leaves. Results of the quantitative analysis showed that plants under drought and salinity stresses produce lower quantity of alkaloids, flavonoids, and tannins while saponin production was increased.

Keywords: *Acalypha wilkesiana*, growth, secondary metabolites, environment, drought, salinity

1. Introduction

Acalypha wilkesiana (Muell. Arg.) Fosberg belongs to the family Euphorbiaceae. It is an ornamental plant which is commonly used for hedging in many parts of the world (Jekayinfa et al., 1997). The plant is known to have variety of ethnomedical uses such as treatments of skin rashes in babies and anti-bacterial effects (Gills, 1992; Spelman et al., 2006). The juice or boiled decoction of the plant is used for the treatment of gastrointestinal disorders and skin infections caused by pathogenic fungi such as *Pityriasis versicolor, Impetigo contagiosa, Candida intetrigo, Tinea versicolor, Tinea corporis* and *Tinea pedis* (Ogundaini, 2005). In addition, the effectiveness of this plant in the management of hypertension has been documented (Ikewuchi et al., 2008). The use of several plant species, having medicinal properties, to cure or prevent illness antedates civilization. The drugs used in modern societies are the fruits of research and development often performed by pharmaceutical companies. Almost all of the valuable drugs are derived from the naturally occurring plant raw materials. At present, in spite of technological developments in chemistry and related fields, plant-derived orthodox drugs still prevail compare to those of synthesis. The medicinal value of these plants is the attribute of some chemical substances that explicate specific physiological effects on humans. Among the most important bioactive compounds, the role of alkaloids, tannins, flavonoids and phenolics has been widely known (Hills, 1952).

Plants grown in given habitat are exposed to various abiotic stresses that may have significant effects on their growth and productivity. Environmental factors such as light, water as well as salinity are important variables affecting phytochemical production in plants (Perez-Balibrea et al., 2008). Drought and salinity rank high as environmental constraints limiting plant productivity and distribution. When plants are exposed to drought stress, they exhibit a wide range of responses in the entire plant, both at cellular and molecular levels (Chaves et al., 2003). More specifically, drought can lead to a series of morphological, physiological and biochemical changes in plants, which in turn adversely affect their growth and productivity. Concerning the salinity, the negative influences on plants *via* photosynthesis inhibition (Sharma et al., 2005), ion toxicity (Patel & Pandey, 2008), and water deficit (Suarez & Medina, 2008) have been demonstrated.

Major processes in plants, such as photosynthesis, protein synthesis, and lipid metabolism, are affected when plants experience the aforementioned abiotic stresses (Parida & Das, 2005). Carbohydrates, which among other substrates are essential for cell growth, are produced *via* photosynthesis and the latter is significantly reduced under stress conditions (Zobayed et al., 2007).

With the increase in the popularity of plant-based medicines including herbal products, phyto-pharmaceuticals and traditional pharmaceuticals derived from plants have opened up a new segment in horticultural crop

production. Nevertheless, optimized agricultural practices have not been developed for many medicinal plants (Murch et al., 2003). It is a common knowledge that the products of these medicinal plants are often rated according to their efficacies, which in turn depends on the concentration of the bioactive compounds or the secondary metabolites. Since environmental factors have direct effects on plant growth and development, it could be validly argued that the synthesis of the bioactive compounds could also be affected by environmental factors (Murch et al., 2000).

Climate change will place new demands on agriculture globally. Emissions of greenhouse gases will result in the warming of the climate across the world and this will likely result in extreme weather events, such as heat waves and drought (Odjegba & Adeniyi, 2012). Whether climate change will increase or decrease the production of secondary metabolites in medicinal plants is yet to be ascertained, since most past works done on phytochemical production did not incorporate the influence of environmental factors. It is therefore the aim of this study to assess the potential impact of two common abiotic stresses on the production of phytochemicals in *Acalypha wilkesiana.*

2. Materials and Methods

2.1 Preliminary Screening of A. wilkesiana for Phytochemicals

Fresh leaves of *A. wilkesiana* were collected from the Botanical Garden of University of Lagos, Akoka. Samples were rinsed in a running tap water and air dried at room temperature in the laboratory. The phytochemical screening was done according to methods described by Harbone (1973), Sofowora (1980), and Trease and Evans (1989). The samples were screened for alkaloids, cardial glycosides, flavonoids, saponins, steroids, and tannins.

2.2 Plant Material and Treatments

Stem cuttings of *A. wilkesiana* were collected from the Botanical Garden, University of Lagos in a single batch and enough for the study. Stems were cut into equal length of 15 cm before planting in nursery bags filled with loamy soil. The nursery material was maintained for 12 weeks to allow sprouting and growth of plants. After this growth period, plants were grouped into 3 and replicated 10 times before subjecting them to 4 weeks of treatments. Group 1 plants served as the control and received 300 ml of water every 3 days for the duration of the experiment. Group 2 plants were subjected to simulated drought for the same period; while group 3 plants received 300 ml of 0.1 M NaCl solution every 3 days for the 4 week period. At the end of the treatment period, plants were harvested and subjected to the quantification of phytochemical substances.

2.3 Quantitative Analyses of Phytochemicals

2.3.1 Alkaloid

Total alkaloid was quantified according to the method of Harborne (1973). About 3 g of the sample was weighed into a beaker and 100 ml of 10% acetic acid in ethanol was added and allowed to stand for 3 h. This was filtered through Whatman no. 1 filter paper and the extract was heated for 1 h on a water bath. Thereafter, 1 M ammonium hydroxide was added drop by drop to the extract until the precipitation was completed. The precipitate, composed of alkaloid, was collected and washed with 0.1 M ammonium hydroxide. It was dried at room temperature and weighed.

2.3.2 Total Flavonoid

The total flavonoid content of the sample was determined using ammonium chloride colorimetric method as described by Chang et al. (2002) with slight modifications. About 1 g of plant samples were extracted with 30 ml of 80% methanol. Extract (1 ml) was separately mixed with 1 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 1 ml of distilled water. The mixture was allowed to stand at room temperature for 45 min. The absorbance of the mixture was measured at 415 nm using a spectrophotometer. The calibration curve was prepared by using Quercetin as standard at final concentrations of 0.0 to 8.0 µg/ml.

2.3.3 Saponin

This was quantified according to the method described by Obadoni and Ochuko (2001) with little modification. About 3 g of dried plant sample was extracted with 30 ml 20% ethanol after heating in a water bath for 3 h with continuous stirring at 60 ºC. After filtration, the residue was re-extracted with another 30 ml 20% ethanol. The extracts from the 2 cycles were combined and heated for 2 h in a water bath at about 80 ºC to reduce the volume to about 15 ml. The concentrate was transferred into a separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. About 20 ml of n-butanol was added and the mixture washed twice with 10 ml of 5% aqueous sodium chloride. After evaporation, the samples were dried in the oven at 65 ^{o}C to a constant weight; the saponin content was calculated

as percentage.

2.3.4 Tannin

Tannin was quantified according to Van-Burden and Robinson (1981). About 0.5 g of the sample was weighed into a 50 ml plastic bottle. Subsequently, 50 ml of distilled water was added and the final volume was shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was read at 120 nm after 10 min of incubation.

2.4 Statistical Analysis

Each analysis was conducted three times. Numerical data were analyzed using one way analysis of variance (ANOVA) and the results presented as mean ± SE.

3. Results

3.1 Qualitative Analysis

The results of the preliminary screening of *A. wilkesiana* for the content of phytochemicals are shown in Table 1. The qualitative analysis revealed the presence of alkaloid, cardial glycosides, flavonoids, saponins, steroids and tannins in the leaves of *A. wilkesiana.* The results showed a high alkaloid content while those of cardial glycosides and steroids were present to a lesser extent.

Table 1. Qualitative analysis of phytochemicals in *Acalypha wilkesiana*

S/N	Phytochemical	Test	Status
1	Alkaloid	Mayer's	+++
2	Cardial glycosides	Keller-Kiliani Test	+
3	Flavonoids	Ammonia solution Test	++
4	Saponin	Frothing Test	++
5	Steroids	Liebermann-Burchard Reaction	+
6	Tannins	$FeCl_3$ Test	++

+++ = high presence, ++ = moderate presence, + = low presence.

3.2 Quantitative Analysis

3.2.1 Effects of Drought and Salinity on Alkaloid Production

Data showing the effects of simulated drought and salinity on alkaloid production in *A. wilkesiana* are presented in Figure 1. As shown in the figure, simulated drought as well as 0.1 M NaCl significantly reduced alkaloid production. The control plants had a mean alkaloid content of 8.813 ± 0.054 mg g^{-1} dry weight. Plants that were exposed to drought and salinity respectively had 3.355 ± 0.08 and 2.644 ± 0.06 mg g^{-1} dry weight. The result showed that salinity had a more severe effect on alkaloid production compared to drought.

3.2.2 Effects of Drought and Salinity on Flavonoid Production

The total flavonoid content in the leaves of *A. wilkesiana* after drought and salinity treatments is shown in Figure 2. A significant difference ($p < 0.05$) exists between the control and the treated plants as both drought and salinity severely affected the flavonoid synthesis. Plants exposed to drought had a mean value of 0.1497 ± 0.01 mg g^{-1} dry weight, while plants treated with 0.1 M NaCl had the least flavonoid content with a mean value of 0.103 ± 0.008 mg g^{-1} dry weight. The control plants however had a mean value of 0.8307 ± 0.02 mg g^{-1} dry weight.

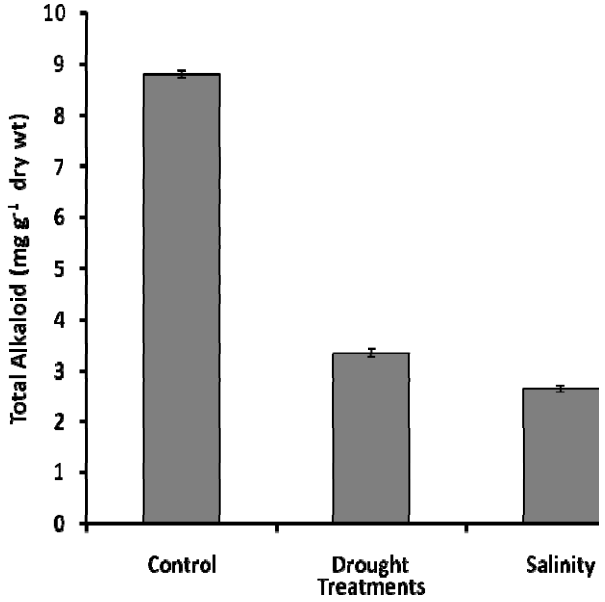

Figure 1. Total alkaloid content in the leaves of *A. wilkesiana* after drought and salinity treatments. Mean and standard error of 3 replicates are presented

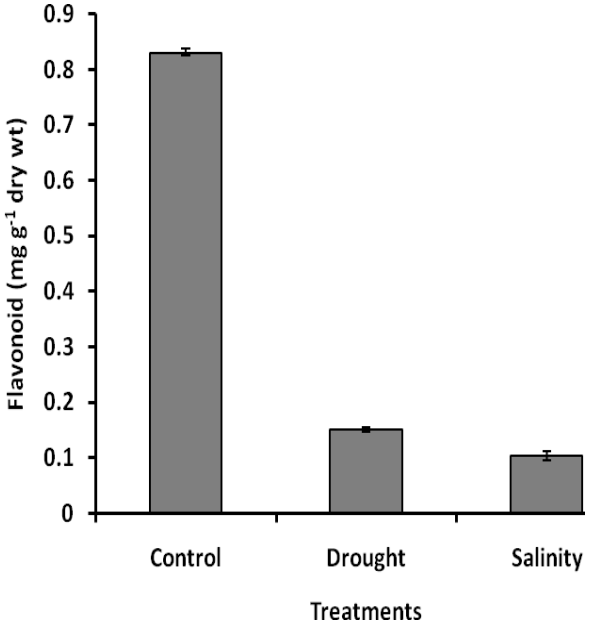

Figure 2. Total flavonoids content in the leaves of *A. wilkesiana* after 4 weekexposure to drought and salinity. Mean and standard error of 3 replicates are presented

3.2.3 Effects of Drought and Salinity on Saponin Production

In this study, it was observed that drought and salinity treatments enhanced saponin production in *A. wilkesiana*. While the control plants had a mean value of 1.283 ± 0.0327 mg g^{-1} dry weight, plants exposed to drought and salinity had mean values of 3.455 ± 0.325 and 1.438 ± 0.0483 mg g^{-1} dry weight, respectively (Figure 3).

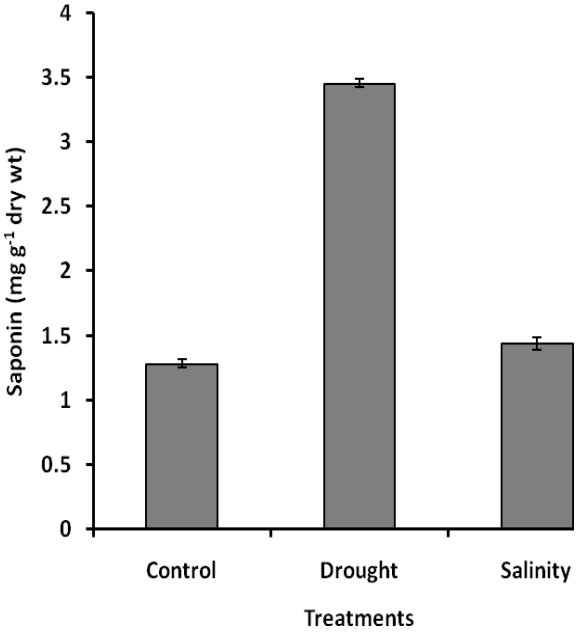

Figure 3. Effects of drought and salinity on the saponin production in *A. wilkesiana* leaves. Mean and standard error of 3 replicates are presented

3.2.4 Effects of Drought and Salinity on Tannin Production

The effects of simulated drought and salinity treatments on tannin production in *A. wilkesiana* leaves are reported in Figure 4. It was observed that stress treatments significantly reduced the tannin production. It is worth to note that salinity hindered tannin production to the higher extent than the drought. Indeed, the mean value of tannin in plants exposed to salinity stress was significantly lower than the value observed for drought-stressed plants. While the control plants had a mean value of 2.0087 ± 0.081 mg g^{-1} dry weight, plants that were exposed to drought and salinity respectively had mean values of 1.141 ± 0.0441 and 0.5737 ± 0.0624 mg g^{-1} dry weight.

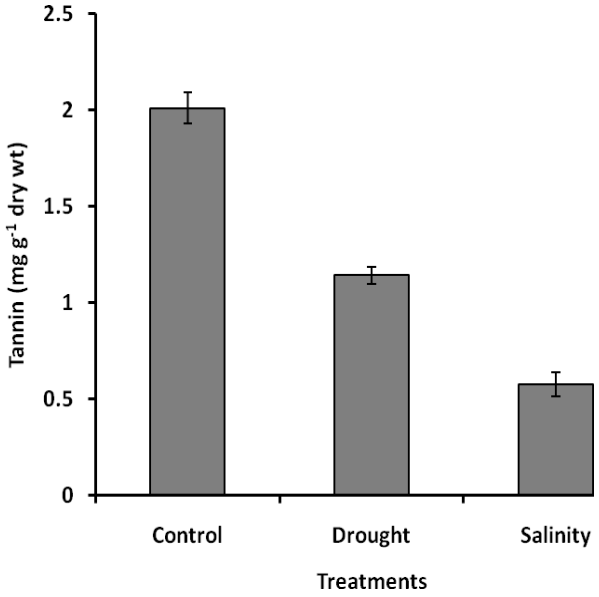

Figure 4. Effects of drought and salinity on the tannin production in *A. wilkesiana* leaves. Mean and standard error of 3 replicates are presented

4. Discussion

The phytochemical screening as well as the quantitative analysis revealed the presence of alkaloids, cardial glycosides, flavonoids, saponins, steroids and tannins in the leaves of *A. wilkesiana*. The results are in agreement with the findings reported by Madziga et al. (2010). Environmental stresses such as drought and salinity can induce generation of reactive oxygen species (ROS) due to the disruption of cellular homeostasis (Sharma & Dubey, 2005; Tanou et al., 2009).

The low levels of total alkaloids, flavonoids and tannins observed in plants grown under drought and salinity treatments could be as a result of ROS effects on enzymes essential for the biosynthesis of these metabolites (Sharma et al., 2012). Similar result was reported by Murch et al. (2003) on *Hypericum perforatum* exposed to nickel. When plants are subjected to drought, ROS production is induced in many ways. Inhibition of carbon dioxide (CO_2) assimilation, together with the changes in photosystem activities and photosynthetic transport capacity under drought stress results in accelerated production of ROS via the chloroplast Mehler reaction (Asada, 1999). Salinity stress results in an excessive production of ROS (Tanou et al., 2009). High salt concentrations enhanced overproduction of ROS by impairment of cellular electron transport within different subcellular compartments such as chloroplasts and mitochondria, also from induction of metabolic pathways such as photorespiration. Salinity can also lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation which subsequently exposed chloroplasts to excessive excitation energy and overreduction of photosynthetic electron transport system leading to enhanced production of ROS and induced oxidative stress. Low chloroplastic CO_2/O_2 ratio also favors photorespiration leading to increased production of ROS such as hydrogen peroxide (Hernandeg et al., 2000).

It was observed in this study that drought and salinity increased saponin content in the plant. This observation agreed with the results reported by De Costa et al. (2013) that saponin content in *Quillaja brasiliensis* leaves increased significantly when exposed to salinity. El-Sayed et al. (2008) also reported that saponin content in *Trubulus* increased when subjected to water stress. This increase could be related to its protective role against oxidative stress (Lin et al., 2009).

There was high presence of alkaloid in *A. wilkesiana* leaves detected. Alkaloid is well known for its toxicity against cells of external organisms and its potential in eliminating human cancer cell lines has been implicated (Nobori et al., 1994). Cardial glycoside, another secondary metabolite present in *A. wilkesiana*, has therapeutic effects on heart-related ailments (Gills, 1992). Flavonoid has been implicated in a wide range of biological activities, such as anti-inflammatory, anti-angionic, anti-allergic, antimicrobial and antioxidant properties (Hodek et al., 2002). The inhibitory effect of saponin on inflamed cells has been documented (Just et al., 1998). Saponin was found to be moderately present in *A. wilkesiana*, and usefulness of this plant in managing inflammation could be related to the presence of this phytochemical (Gills, 1992). The presence of steroid in *A. wilkesiana* is of interest due to its implication in various anabolic hormones including sex hormones (Okwu, 2001). Plants containing tannins as one of its main phytochemicals are astringent in nature and are used to treat intestinal disorders (Dharmananda, 2003). This observation correlates positively with the use of *A. wilkesiana* in herbal medicine. It was observed by Li et al. (2003) that tannins also have anticancer activity and can be used to prevent cancer. This therefore suggests that *A. wilkesiana* is a potential source of bioactive compounds for the treatment and prevention of cancer.

The presence of these phytochemicals in high concentrations in *A. wilkesiana* qualifies it as a potential source of useful drugs in the future. The compounds are known to be biologically active and thus aid antimicrobial activities of the plant (Sofowora, 1993). It was observed in this study that simulated drought as well as salinity treatments significantly reduced the production of the phytochemicals except saponins, suggesting that these environmental stresses may affect the quantity of phytochemicals in medicinal plants and that *A. wilkesiana* is susceptible to these stresses. Considering the importance of medicinal plants in ethnomedicine as well as sources of modern day drugs in pharmaceutical companies, scientists should begin to work towards fortifying medicinal plants genetically to enable them tolerate certain levels of drought and salinity which would ordinarily affect the synthesis of phytochemicals in these plants.

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