THE INFLUENCE OF WATER STRESS (DROUGHT) ON THE ALKALOID, PHENOL AND STEROID CONTENT OF THE LEAVES OF *OCIMUM Gratissimum (L.)* AND *Gongronema latifolium* (BENTH)

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Abstract
The influence of water stress on the alkaloid, phenol and steroid content of the leaves of Ocimum gratissimum and Gongronema latifolium was investigated. Cultivated O. gratissimum and G. latifolium plants in plastic planting buckets were subjected to mild water stress by supplying 500 ml of water to each planting bucket once in a week, while the control planting buckets were each supplied with 750 ml of water thrice a week. Treatment started two months after seedling emergence and occurred for one month. The leaves of the plants were harvested here after for analysis. The leaf was used for the analysis, since it is the most eaten part. Water stress significantly (P< 0.05) increased the alkaloid content of the leaves of O. gratissimum and G. latifolium. On the other hand, the phenols and steroids content of the leaves of the two plants studied was significantly (P < 0.05) reduced by water stress. The importance of these findings is that water stress enhanced the concentration of alkaloids in the leaves of the plants, which is medicinally important but negatively affected the concentration of phenols and steroids that have pharmaceutical values.

Keywords: alkaloids, phenols, steroids, water stress, *Ocimum gratissimum* and *Gongronema latifolium*

Introduction

The importance of plants in the maintenance of good health cannot be over emphasized. Research reports have indicated the role of plants in maintenance of good health (Ijeh *et al*. 2004; Osuagwu 2008; Osuagwu and Nwosu 2006; Edeoga and Eriata 2001; Gill 1992). WHO (1990), also had earlier on observed that the majority of the population in the developing countries still rely on herbal medicine to meet their health need. In Nigeria, these plants constitute great reservoir of wide variety of compounds, which exhibit some medicinal and nutritive properties (Edeoga *et al*. 2003; Osuagwu *et al*. 2007; Okwu 2001, 2004), hence therefore, are used as spices, food, or medicinal plants.

Many of these indigenous plants contain bioactive compound that cause physiological activities against bacteria and other microorganisms and are precursor for the synthesis of useful drugs (Okwu 2001; Sofowora 1993). The usefulness of these plant materials medicinally, is due to the presence of bioactive constituents such as alkaloids, tannins, flavonoids, steroids and phenolics compounds (Hill, 1952)..These chemicals are known to carry out vital medicinal roles in human body.

Alkaloids are known to have a powerful effect on animal physiology.

They play some metabolic role and control development in living system (Edeoga and Eriata, 2001). They are also used as starting materials in the manufacture of steroidal drugs and carry out protective function in animals, thus are used as medicine especially steroidal alkaloids (Maxwell *et al*., 1995; Stevens *et al*., 1992). Isolated pure plant alkaloids and their synthetic derivatives are used as basic medicinal agent for their analgesic, antispasmodic and bacteridal effect (Ogukwe, *et al*., 2004). Phenolic compounds in plants are potentially toxic to the growth and development of pathogens (Singh and Sawhney, 1988). Research reports also show that phenolic compounds carry out potent antioxidant activity and wide range of pharmacologic activities which include anti- cancer, antioxidant and platelet aggregation inhibition activity (Frankel *et al*., 1993; Rein *et al*., 2000; Rice – Evans *et al*., 1996; Wattenberg *et al*., 1980). Steroid containing compounds are of importance in pharmacy due to their role in sex hormones (Okwu, 2001).Steroids such as equine estrogen are implicated in the reduction or risks of coronary heart and neurodegenerative diseases in healthy and young post menopausal women (Perrella *et al*., 2003). These plants are therefore used for the treatment of many diseases such as rheumatism, diarrhea, malaria, elephantiasis, cold, obesity, dysentery, high blood pressure, malnutrition, gonorrhea and others (Anigbogu and Uzoaga 2006; Morebise *et al*. 2002; Burkill 1995; Gill 1992).

The biosynthesis of these bioactive plant chemicals is influenced by various agronomic and environmental factors. Water stress (drought) is reported to influence the concentration of phytochemicals in plants (Ghaderi *et al*. 2006 Selmar 2008; Zheng *et al*. 2006). The concentration of alkaloid in the tissues of plants has been reported to be affected by water stress. Increased alkaloids in plants as a result of water stress was reported by researchers (Hoft *et al*., 1996; Li and Yan, 2007; Selmar , 2008). However, Paul *et al*. (2006), observed that water stress had no significant effect on the alkaloid content of *Rauvolfia serpentina*. The phenolic compounds contented of plants were also showed to be affected by water stress. Research reports indicated that water stress generally caused significant reduction in the phenolics compound of plants (Chakraborty *et al*., 2002; Zheng *et al*., 2006; Cherviyot *et al*., 2007). On the other hand, Hura *et al*., (2008) and Selmar (2008), observed significant increase in the phenol content of plants due to water stress. The phenolics compounds in the leaves protect the mesophyll cells by absorbing light reaching the leaves and transforming it into blue fluorescence, thus limiting the excitation of the chlorophyll during water stress (Hura *et al*., 2008).

Ocimum gratissimum a dicotyledonous shrub plant belongs to lamiaceae family. Its use in the treatment of many diseases such as upper respiratory infection, diarrhea, pile, cough, fever, pneumonia, surface wound, gonorrhea, worm infestation, stomach ache has been documented (Burkill, 1995; Gill, 1992; Iwalokun et al., 2003; Nangia-Makker et al., 2007; Okeke, 1998). It is also implicated in blood coagulation and renal function (Anigbogu and Uzoaga, 2006; Edemeka and Ogwu, 2001). The leaves of O. gratissimum are used to prepare soup and porridge for women after delivery among the Igbos of Nigeria (Ijeh et al., 2004) and are also

used as spices for preparation of food (Burkill, 1995; Ijeh et al., 2004). The leaf extracts are used as insect repellants (Bekele et al., 1996; Ogendo et al 2008).

G. latifolium belongs to the Asclepiadaeae family. It is a slender climber often 3-4 metres long. Leaves are simple with blade that is ovate with cordate base and acuminate apex. The margin of the leaf is entire with petiole that is up to 4 cm long (Grubben and Denton, 2004). Its use in the treatment and cure of cough, loss of appetite, diabetes, malaria, improved liver function and asthma have been reported (Burkill, 1985; Morebise *et al*., 2002; Nwanjo and Alumanah, 2006; Nwosu and Malize, 2006; Okeke and Elekwa, 2006; Oshinubi *et al*.,2006; Ugochukwu *et al*,2003). The leaves and seeds are used as spices or condiments in the diet of nursing mothers and they are also used raw as salad and to flavour meat preparations and fresh fish pepper soup (Nwosu and Malize, 2006; Okafor *et al*., 1996).

This research investigated the effect of water stress on the potential of alkaloids, phenols and steroids in the leaves of *O. gratissimum* and *G. latifolium* and to determine if water stress enhanced or reduced the concentration of these phytochemicals in view of their importance in medicine and in pharmaceutical industries.

Materials and Methods

Plant sample

The seeds of O. gratissimum were collected from a home stead in Amaogwu village, Bende Local Government Area of Abia State. The fresh and succulent stem cuttings of G. latifolium was obtained from the forest strip of the Forestry Department College of Natural Resources and Environmental management, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. Both plant materials were identified by the Taxonomic unit of the Botany section of the Biological Sciences Department, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. The seeds of O. gratissimum were raised into seedling in nursery boxes before they were transplanted into planting buckets. Stem cuttings of G. latifolium were planted directly into the planting buckets.

Cultivation of the plants was carried out using 40 plastic planting buckets containing 8 kg of sterilized soil. Treatment consists of two levels of treatments mild stress (non-irrigated) and control (irrigated) in 10 replicates. In the water stress treatment, 500 ml of water was supplied to each planting bucket once in a week, while in the control treatment; 750 ml of water was supplied to each planting bucket thrice in a week. Treatment commenced two months after seedling emergence and was carried out for one month. The leaves were then harvested for analysis.

The research was carried out in the Green House of the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State.

Preparation of leaves for analysis

The leaves of the plants were collected for analysis. The leaves were used due to the fact that they are the part normally used for preparing meals. The harvested leaves were oven dried using Selecta Model 150-9001 oven at 65oC for 24 hours and ground into powder using Thomas Willey Milling Machine. Powdered samples were stored in sample

bottles and kept in a dry place to be used for analysis.

Qualitative analysis for the presence of alkaloids, phenols and steroids Test for the presence of alkaloids

The presence of alkaloids in each sample was investigated using the method described by Harborne (1973).

An alcoholic extract was used and obtained by dispersing 2g of the powered sample in 10mls of ethanol. The mixture was through shaken before filtering using Whatman No (40) filter paper. 2ml of the filtrate was added into a test tube and 3 drops of pirovic acid was mixed with it. The formation of light green colouration indicates presence of alkaloid.

Test for the presence of phenols

The presence of phenols in the sample was carried out using the Harborne (1973) methods.The fat free sample was boiled with 50ml of ether for 15 minutes. 5ml of the extract was pipette into a 50ml flask and 10ml of distilled water added into it. 2ml of ammonia hydroxide solution and 5ml of concentrated amyl alcohol were also added. The mixture was allowed to react for 30 minutes for colour development.

Test for the presence of steroid

The presence of steroid in the test sample was carried out using the method described by Harborne (1973).2ml of acetic anhydride was added to 5ml ethanol extract of each sample with 2ml sulphuric acid (H_2SO_4). The colour changed from violet to blue or green in the sample indicates the presence of steroids.

Quantitative determination of alkaloids, phenols and steroids. Alkaloid determination

The determination of the concentration of alkaloid in the leaves of *O .gratissimum* and *G. latifolium* was carried out using the alkaline precipitation gravimetric method described by Harborne (1973).5g of the powdered sample was soaked in 20ml of 10% ethanolic acetic acid. The mixture was stood for four (4) hours at room temperature. Thereafter, the mixture was filtered through Whatman filter paper (No 42). The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60^oC for 30 minutes, cooled in a dessicator and reweighed. The process was repeated two more times and the average was taken. The weight of alkaloid was determined by the differences and expressed as a percentage of weight of sample analyzed as shown below.

$$\%Alkaloid = \frac{W_1 - W_2}{W_s} \times \frac{100}{1}$$

Where:-
W_1 = Weight of filter paper
W_2 = Weight of filter paper + alkaloid precipitate
W_s=Weight of Sample

Determination of phenols

The concentration of phenols in the leaves of *O. gratissimum* and *G.*

latifolium was determined using the folin- cio Caltean colorimetric method described by Pearson (1976). 0.2 g of the powdered sample was added into a test tube and 10ml of methanol was added to it and shaken thoroughly the mixture was left and to stand for 15 minutes before being filtered using Whatman (No42) filter paper. I ml of the extract was placed in a text-tube and I ml folin-cio Caltean reagent in 5ml of distilled water was added and color was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760 nm wave. The process was repeated two more times and an averaged taken. The phenol content was calculated thus:

% Phenol =100 /w x AU /AS x C/100x VF/ VA x D

Where,

W= weight of sample analyzed
AU= Absorbance of test sample
AS= Absorbance of standard solution
C= concentration of standard in mg/ml
UF= total filtrate volume.
VA= Volume of filtrate analyzed
D= Dilution factor were applicable

Steroid determination

The steroid content of the leaves of the plants was determined using the method described by Harborne (1973). 5g of the powdered sample was hydrolysed by boiling in 50 ml hydrochloric acid solution for about 30minutes. It was filtered using Whatman filter paper (N042), the filtrate was transferred to a separating funnel. Equal volume of ethyl acetate was added to it, mixed well and allowed separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The extract was dried at 100^{o}C for 5minutes in a steam bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture becomes turbid and a reweighed Whatman filter paper (N042) was used to filter the mixture properly. The dry extract was then cooled in a dessicator and reweighed. The process was repeated two mere times and an average was obtained.

The concentration of steroid was determined and expressed as a percentage thus:

$$\%Steroid = \frac{W_1 - W_2}{W_s} \times \frac{100}{1}$$

Where,
W_1= weight of filter paper.
W_2 = weight of filter paper + steroid
W_s=Weight of Sample

Statistical Analysis

The design for this study was complete randomized design in ten replicates of each treatment. The normal paired t-test at 0.05 probability level was used to analyse the data to determine significant level between treatments.

Results and Discussion

The results of the effect of water stress on the alkaloid, phenol and steroid content of the leaves of *Ocimum gratissimum* and *Gongronema latifolium* are summarized in tables 1 to 4.

The presence of alkaloids, phenols and steroids in the leaves of *O. gratissimum* and *G. latifolium* was not affected by water stress. Leaf samples of both stressed and unstressed plants showed presence of these phytochemicals (tables1 and 2). This

observation might be due to the fact that these phytochemicals are natural constituents of the leaves of *O. gratissimum* and *G. latifolium* and water stress did not affect their presence. These compounds have earlier on been found in the leaves of *O. gratissimum* and *G. latifolium* (Ijeh *et al*., 2004; Okeke and Elekwa, 2003).

Water stress significantly (P<.0.05) increased the concentration of alkaloids in the leaves of *O. gratissimum* and *G. latifolium* (tables3 and 4). The observed increase in the alkaloid content as a result of water stress might be due to increased accumulation of metabolites by stressed plants to withstand the effect of stress (Stewart *et al*., 2007; Gu *et al.*, 2008). Increased concentration of alkaloid in other plants as a result of water stress was reported by other researchers (Hoft *et al*., 1996; Lin *et al*., 2007; Selmar, 2008). The concentration of phenols in the leaves of *O. gratissimum* and *G. latifolium* was significantly (P < 0.05) reduced by water stress (tables 3 and 4). Reduced concentration of phenols of plant tissues was also observed by other workers (Cheviyot *et al*., 2007; Zheng *et al*., 2006). On the other hand, Hura *et al*., (2008) and Selmar, (2008), reported increased concentration of phenols in plants as a result of effect of water stress. The reduction of phenols in stressed plants might be due to their breakdown during water stress as a means of with standing the effects of water stress. Water stress significantly (P < 0.05) caused reduction in the steroid content of the leaves of *O. gratissimum* and *G. latifolium* (tables 3 and 4). This reduction might be associated with the breakdown of certain materials in plants in response to water stress. The importance of these findings is that water stress will enhance the production of alkaloids and lower the accumulation of phenols and steroids which are of high medicinal and pharmaceutical value.

We conclude that water stress tends to have both negative and positive influence on the accumulation of these phytochemicals, hence affecting their efficacy and medicinal value.

Table 1: The influence of water stress (drought) on the presence of alkaloid, phenol and steroid in the leaves of *Ocimum gratissimum*

Treatment	alkaloid	phenol	steroid
Unstressed plants	+	+	+
Stressed plants	+	+	+

+ = Present

Table 2: The influence of water stress (drought) on the presence of alkaloid, phenol and steroid in the leaves of *Gongronema latifolium*

Treatment	alkaloid	phenol	steroid
Unstressed plants	+	+	+
Stressed plants	+	+	+

+ = Present

Table 3: The influence of water stress (drought) on the percentage alkaloid, phenol and steroid content of the leaves of *O. gratissimum*

Treatment	alkaloid	phenol	steroid
Unstressed plants	0.020 ± 0.0012	0.395 ± 0.0012	0.119 ± 0.0591
Stressed plants	0.030 ± 0.0014	0.334 ± 0.0015	0. 040 ± 0.0014
Mean SE	± 0.0024	± 0. 0023	± 0. 0595

SE = standard error

Table 4: The influence of water stress (drought) on the percentage alkaloid, phenol and steroid content of the leaves of *G. latifolium*

Treatment	alkaloid	phenol	steroid
Unstressed plants	0. 006 ± 0.0002	0.364 ± 0.0021	0.080 ± 0. 0017
Stressed plants	0. 012 ± 0. 0006	0.303 ± 0.0020	0.060 ± 0. 0012
Mean SE	± 0. 0006	± 0.0031	± 0. 0025

SE = standard error

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