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Effect of Water Stress (Drought) on the Antimicrobial Activity of the Leaves of *Ocimum gratissimum* L. and *Gongronema latifolium* Benth.

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ABSTRACT

The effect of water stress (drought) on the antimicrobial activity of the leaves of *Ocimum gratissimum* L. and *Gongronema latifolium* Benth. was investigated. Cultivated *O. gratissimum* and *G. latifolium*, planted in buckets, were subjected to mild stress by supplying each bucket with 500 ml of water once a week, while the control treatment was supplied with 750 ml of water three times a week. The treatment commenced 2 months after seedling emergence. Leaves were harvested for the sensitivity test one month from the date of commencement of treatment. The sensitivity test of the ethanolic extracts of the harvested leaves was applied to *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger.* Water stress (drought) significantly ($P < 0.05$) reduced the antimicrobial activity of *O. gratissimum* and *G. latifolium* leaves except for *Candida albicans*, whose growth reduction was not significant. There was a decrease in the inhibition zone due to water stress treatment. The ethanolic extracts of the leaves of both plants had no antimicrobial effect on *A. niger.* Water stress (drought) reduced the bioactive substances in the leaves which in turn affected the ability of the extracts to inhibit pathogenic activity, thus affecting the efficacy of the plants as medicinal plants.

Keywords: ethanolic extract, inhibition zone, innocula, pathogens

INTRODUCTION

The importance of plants in the maintenance of good health cannot be over emphasized. Research reports have indicated the role of plants in the maintenance of good health (Gill 1992; Edeoga and Eriata 2001; Ijeh *et al*. 2004; Osuagwu and Nwosu 2007; Osuagwu 2008). WHO (1990) had also earlier observed that the majority of populations in developing countries still rely on herbal medicine to meet their health needs. In Nigeria, these plants constitute a great reservoir of a wide variety of compounds that exhibit some medicinal and nutritive properties (Okwu 2001; Edeoga *et al*. 2003; Okwu 2004; Osuagwu *et al*. 2007; Edet *et al*. 2009; Latha and Reddy 2009; Akuodor *et al*. 2010); therefore, they are used as spices, food, or medicinal plants.

Many of these indigenous plants contain bioactive compounds that have physiological activity against bacteria and other microorganisms and are precursors for the synthesis of useful drugs (Sofowora 1993; Okwu 2001). The antimicrobial activities of these plants and their products have been widely reported (Okeke 1998; Ijeh *et al*. 2005; El-Zaher *et al*. 2006; Mevy *et al*. 2007; Sahraoui *et al*. 2007; Vagionas *et al*. 2007; Nwinyi *et al*.2009; Sengui *et al*. 2009; Pirbalouti *et al*. 2010). 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 (Gill 1992; Burkill 1995; Morebise *et al*. 2002; Anigbogu and Uzoaga 2006; Edet *et al*. 2009; Akuodor *et al*. 2010).

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 (Bertamini *et al*. 2006; Ghaderi *et al*. 2006 Selmar 2008; Zheng *et al*. 2006; Khalid and Teixeira da Silva 2010; Osuagwu and Edeoga 2010; Osuagwu *et al*. 2010a; Osuagwu *et al*. 2010b ; Osuagwu *et al*. 2010c).

The antimicrobial activity of *Ocimum gratissimum* (Iwalokun *et al*. 2003; Ezekwesili *et al*. 2004; Ijeh *et al*. 2005; Tchoumbougnang *et al.* 2005; Matasyoh *et al*. 2007; Mbata and Salkia 2007; Nweze and Eze 2009; Nwinyi *et al*. 2009; Oboh *et al*. 2009; Prabhu *et al*. 2009) and *Gongronema latifolium* (Okeke and Elekwa 2006; Afolabi and Eleyinmi 2007; Nwinyi *et al*. 2008) are well documented.

The objective of this study was to determine the implication of water stress (drought) on the antimicrobial activity of the leaves of *O. gratissimum* and *G. latifolium*.

MATERIALS AND METHODS

Plant sample

The seeds of *O. gratissimum* were collected from a homestead 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 seedlings in nursery boxes before being transplanted into planting buckets. Stem cuttings of *G. latifolium* were planted directly into planting buckets.

Plants were cultivated in 20 plastic planting buckets containing 8 kg of sterilized soil each. Treatments consisted of two levels: mild stress (non-irrigated) and control (irrigated) in 10 replicates per treatment. In the water stress treatment, 500 ml of water

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was supplied to each planting bucket once a week while in the control treatment; 750 ml of water was supplied to each planting bucket three times a week. Treatment commenced 2 months after seedling emergence and was carried out for one month. Leaves were then harvested for the antimicrobial activity test. The research was carried out in the greenhouse of the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State.

Determination of antimicrobial activity

1. Preparation of plant extract

The ethanolic extracts of the leaves of *O. gratissimum* and *G. latifolium* was prepared using the method of Ijeh *et al.* (2005).

Powdered samples (50 g) was soaked in 200 ml of absolute ethanol and allowed to stand for 24 h. This was filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness over a steam bath. The residue was dissolved in deionized water to obtain the desired plant extract for the antimicrobial tests.

2. Preparation of innocula

Klebsiella pneumoniae, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger* used in the research were obtained from stock cultures of the Microbiology Laboratory, Federal Medical Centre, Umuahia, Abia State, Nigeria. A viability test for each isolate was carried out by resuscitating the organisms in buffered peptone broth and thereafter sub-culturing into nutrient agar medium and incubating at 37°C for 24 h. The bacterial cultures were incubated in darkness, while those of the fungi were in the light.

3. Antimicrobial test

The sensitivity of the test organisms to the ethanolic extracts of the leaves of *O. gratissimum* and *G. latifolium* was carried out using the diffusion method described by Ebi and Ofoefule (1997).

Molten nutrient agar (20 ml) was seeded with 0.2 ml of broth culture of the test microorganisms in sterile Petri dishes, which were rotated slowly to ensure their uniform distribution. Petri dishes were left to solidify and cups 8.0-mm in diameter were made in the agar using a sterile Pasteur pipette. Petri dishes were allowed to stand for about 30 min at room temperature to allow for proper diffusion of the extracts. The plates were then incubated at 37°C for 24 h. The zone of inhibition (in mm) were measured and recorded.

The test was carried out in the laboratory of the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State.

Statistical analysis

The experiment was not a completely randomized block design since only two factors (irrigated and non irrigated) were involved. The water stress experiment was in two treatments of 10 replicates each. 40 plants were used for each set of experiment, 20 per treatment. The antimicrobial test was carried out in triplicate for each sample and significant differences between treatment means were assessed by a normal paired *t*-test using Special Package for Social Sciences (SPSS) version 15.

RESULTS AND DISCUSSION

Water stress (drought) significantly affected the antimicrobial activity of the leaves of *O. gratissimum* and *G. latifolium* (**Table 1**).

Water stress significantly reduced the ability of *O. gratissimum* leaves to inhibit the activity of *K. pneumoniae*, *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi* and *S. faecalis*; the effect on *C. albicans* was not significant (**Table 1**). The antimicrobial activity of the leaves of *G. latifolium* on all microorganisms was significantly decreased by water stress (**Table 1**). This implies that water stress significantly increased the microbial activity of these pathogens, which might be related to a decrease in amount of bioactive substances (alkaloids, phenols, flavonoids, saponins, steroids, vitamins and other plant chemical compounds) as a result of water stress. Water stress negatively affects the photosynthetic apparatus and photosynthesis resulting in a decrease in the production of primary and secondary products in plants (Yordanov *et al*. 2003; Li *et al*. 2007; Stewart *et al*. 2007; **Table 2**). Water stress may cause the breakdown of certain metabolites as a means of withstanding the effects of drought, leading to a reduction in their quantity (Abdel-Kader 2001; Bertamini *et al*. 2006; Toumi *et al*. 2008). A decrease in the concentration of ascorbic acid content in the leaves of *O. gratissimum* and *G. latifolium* due to water stress was observed (Osuagwu and Edeoga 2010; Osuagwu *et al*. 2010a). Osuagwu *et al*. (2010c) also observed that water stress decrease the concentration of saponin in the leaves of both medicinal plants. Saponin and other bioactive substances have antimicrobial properties (Edeoga *et al*. 2009). A reduction in the concentration of phytochemicals, vitamins and other chemicals due to the effect of water stress has also been reported. Cheriviyot *et al*. (2006) and Zheng *et al.* (2006) observed that water stress led to a significant reduction in the phenolic content of tea (*Camellia sinensis*), *Echinaceae purpurea* and *E. angustifolia*. A significant reduction in the flavonoid content of sweet potato and mung beans due to water stress (drought) was reported by Lin *et al*. (2006) and Tawfik (2008), respectively. The essential oil content of the roots of *Atractylodes lancea* and *Anemopsis californica* was significantly reduced by water stress (Medina-Holiguin *et al.* 2007; Gu *et al*. 2008). Furthermore, the ascorbic acid content of *Vigna unguiculata* was significantly reduced by water stress (Manivannan *et al*. 2008; Nair *et al*. 2008). A reduction in the concentration of these bioactive substances might have negatively affected the ability of their leaf extracts to inhibit the activities of the test pathogens.

The pharmaceutical and therapeutic potential of *O. gratissimum* and *G. latifolium* have been documented. The antimicrobial activity of *O. gratissimum* has been noted by several authors (Holetz *et al*. 2003; Iwalokun *et al*. 2003; Ezekwesili *et al*. 2004; Nakamura *et al*. 2004; Adebolu and Oladimeji 2005; Matasyoh *et al*. 2007; Oboh *et al*. 2009; Nwinyi *et al*. 2009; **Table 3**). *O. gratissimum* have been used for ages as folk medicine for the treatment of many diseases such as upper respiratory tract infection, diarrhea, pile, cough, fever, pneumonia, surface wound, gonorrhea, worm infestation and stomachaches (Gill 1992; Burkill 1995; Okeke 1998; Iwalokun *et al*. 2003; Nangia-Makker *et al*. 2007; Nweze and Eze 2009; Prabhu *et al*. 2009). The leaf extracts are used to reduce blood glucose level (Owoyele *et al*. 2005; Mohammed *et al*. 2007). Its role in blood coagulation and renal function has been reported (Edemeka and Ogwu 2002; Anigbogu and Uzoaga 2006). The leaves of *O. gratissimum* are used to prepare soups and porridge for women after delivery among Nigeria's Igbos (Ijeh *et al*. 2004). The leaves are also used as spices in food preparation (Burkill 1995; Ijeh *et al*. 2004).

The leaf extracts of *G. latifolium* have antimicrobial activity (Afolabi and Eleyinmi 2007; Nwinyi *et al*. 2008; **Table 3**). The use of *G. latifolium* in herbal medicine for the treatment of cough, loss of appetite, odoema, diabetes, ulcer, and to improve liver function is well known (Burkill 1985; Morebise *et al*. 2002; Ugochukwu *et al*. 2003; Nwanjo and Alumanah 2006; Okeke and Elekwa 2006; Oshinubi *et al*. 2006; Edet *et al*. 2009; Akuodor *et al*. 2010). Asthmatic patients usually chew fresh *G. latifolium* leaves to relieve wheezing (Nwosu and Malize 2006). Leaves are used as spices or condiment in the diet of nursing mothers and are used raw as salad and to flavour meat preparation and fresh fish pepper soup (Okafor *et al*. 1996; Nwosu and Malize 2006). The leaves are also used locally in beer brewing (Nwosu and Malize 2006).

The microbial activity of *Aspergillus niger* was not affected by the ethanolic extracts of the leaves of both plants,

Table 1 Effect of water stress (drought) treatment on the antimicrobial activity of the leaves of *Ocimum gratissimum* and *Gongronema latifolium* on *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger*.

Treatment	*Ocimum gratissimum*			*Gongronema latifolium*		
	Irrigated	Non irrigated	*t*-Value	Irrigated	Non irrigated	*t*-Value
	Inhibition zone (mm)			Inhibition zone (mm)		
K. pneumoniae	20.00 + 2.30	15.00 + 2.31	4.564**	18.00 + 2.75	16.00 + 2.62	3.464**
E. coli	24.00 + 2.75	20.00 + 2.70	2.657*	22.00 + 2.74	20.00 + 2.94	3.721**
S. aureus	20.00 + 2.45	17.00 + 2.75	2.535*	20.00 + 2.75	17.00 + 2.75	2.714*
P aeruginosa	16.00 + 2.57	13.00 + 2.27	2.683*	15.00 + 2.05	12. 00 + 2.05	3.105*
S. typhi	24.00 + 2.05	20.00 + 2.62	4.045**	21.00 + 2.21	18.00 + 2.30	5.031**
S. faecalis	22.00 + 2.05	18.00 + 2.45	4.472**	20.00 + 2.45	16.00 + 2.05	3.523**
C. albicans	10.00 + 2.06	8.00 + 2.44	2.023^{NS}	10.00 + 2.44	7.00 + 2.00	2.209^{NS}
A. niger	0.00	0.00	0.0	0.00	0.00	0.00

SE = Standard errors; ** Significant at $P < 0.01$; *Significant at $P < 0.05$; NS = Not significant

Table 2 Methods and rates of water stress treatments as described by some authors.

Reference	Method	Type of treatment	Rate of treatment
Li *et al*. 2007	water	Mild drought, severe drought and water logging	Once a week
Stewart *et al*. 2007	water	Complete submersion to severe drought	Once a week
Yordanov *et al*. 2003	water	Mild to severe drought	Once a week
El-Azim *et al*. 2009	water, salt solution	Mild to severe drought	Once a week
Abbaszadeh *et al*. 2008	water	Mild to severe drought	Once a week
Osuagwu *et al*. 2010	water	Mild drought	Once a week

Table 3 The antimicrobial activity of plant extracts as observed by some authors.

Reference	Pathogens	Concentration of extracts	Plant and source of extract
Holetz *et al*. 2003	Trypanosomatid, *Herpetomonas samuelpessoai*	20–250 µg ml^{-1}	*Ocimum gratissimum* (scent plant). Leaves
Iwalokun *et al*. 2003	*Shigella dysenteriae*, *S. flexneri*, *S. sonnei*, *S. boydii*	3,000 µg ml^{-1}	*O. gratissimum* (scent plant) *Mormodica balsamina* (balsam pear), *Terminalia avicennioides* (tropical almond). Leaves
Nakamura *et al*. 2004	*Candida albicans*, *C. krusei*, *C. tropicalis*	Not indicated	*O. gratissimum* (scent plant). Leaves
Adebolu and Oladimeji 2005	*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *S. typhimurium*	Not indicated	*O. gratissimum* (scent plant). Leaves
Matasyoh *et al*. 2007	*S. aureus*, *Bacillus* spp., *E. coli*, *Pseudomonas aeruginosa*, *Proteus murabilis*, *C. albicans*	Not indicated	*O. gratissimum* (scent plant). Leaves
Oboh *et al*. 2009	*E. coli*, *S. aureus*	Not indicated	*O. gratissimum* (scent plant). Leaves
Nwinyi *et al*. 2009	*E. coli*, *S. aureus*	Not indicated	*O. gratissimum* (scent plant), *Piper guineense* (West African pepper). Leaves
Afolabi and Eleyinmi 2007	*Bacillus cereus*, *B. subtilis*, *E. coli*, *Enterobacter aerogenes*, *E. agglomerans*, *E. faecalis*, *Salmonella cholerasius* ser *typhimurium*, *S. aureus*, *Yersinia enterolytica*, *Listeria monocytogenes*	100 mg ml^{-1}	*Gongronema latifolium* (amaranth globe). Leaves
Nwinyi *et al.* 2008	*E. coli*, *S. aureus*	10 mg ml^{-1}	*Pisidium guajava* (guava), *G. latifolium* (amaranth globe). Leaves
Parag *et al* 2010	*E. coli*, *S. typhi*, *Shigella dysenteriae*, *Vibro cholerae*, *Pseudomonas pyrocyaneus*, *Proteus vulgaris*	40 mg ml^{-1}	*Ocimum sanctum* (tulsi). Leaves
Mishra and Mishra 2011	*E. coli*, *P. aeruginosa*, *Salmonella typhimurium*, *S. aureus*	Not indicated	*O. sanctum* (tulsi). Leaves
Joshi *et al*. 2011	*E. coli*, *S. aureus*, *Klebsiella pneumoniae*, *S. typhi*, *P. aeruginosa*, *Salmonella paratyphi*	Not indicated	*O. sanctum* (tulsi), *Azadirachta indica* (neem), *Achyranthes bidentata* (datiwan), *Eugenia caryophyllata* (clove). Leaves

whether stressed or unstressed. This indicates that the extracts of the leaves of *O. gratissimum* and *G. latifolium* have no antimicrobial effect on *A. niger*.

The importance of this study's findings is that water stress has a negative impact on the ability of *O. gratissimum* and *G. latifolium* leaf extracts to inhibit the activity of all microorganisms tested, hence their reduced efficacy and value as medicinal plants. This affects their importance in the control and cure of diseases related to these pathogens.

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