Medicinal and Aromatic Plant Science and Biotechnology ©2011 Global Science Books

Effect of Fertilizer Treatment on the Antimicrobial Activity of the Leaves of *Ocimum gratissimum* L. and *Gongronema latifolium* Benth.

Gabriel Gbenimakor Ejikeme Osuagwu* • **Hillary O. Edeoga**

Biological Sciences Department, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia State, Nigeria

Corresponding author: * **gbekus2002@ yahoo.com**

ABSTRACT

The effect of fertilizer treatment on the antimicrobial activities of the leaves of *Ocimum gratissimum* (L.) and *Gongronema latifolium* (Benth) was investigated. Cultivated *O. gratissimum* and *G. latifolium* were applied with NPK (15:15:15) fertilizer at 100, 200, 300, 400 and 500 kg/ha treatment levels in planting buckets derived using the furrow slice method two months after seedling emergence. No fertilizer treatment served as control. Leaves were harvested one month after treatment. The ethanolic extracts of the harvested leaves were used to determine the sensitivity of the extracts on *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aurues*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger.* The result obtained showed that the antimicrobial activities of the leaves of *O. gratissimum* and *G. latifolium* was significantly ($P < 0.05$) increased by fertilizer treatment. The inhibition zone increased with increase in the level of fertilizer treatment. The ethanolic extracts of both plants whether treated or not had no antimicrobial effect on *A. niger*. This research revealed that fertilizer treatment might have increased the phytochemical content of the leaves of the plants which in turn enhanced their antimicrobial potential. These phytochemicals are known to exhibit physiological activities against bacteria and other microorganisms.

Keywords: bioactive compounds, ethanolic extracts, inhibition zone, pathogens
Abbreviations: NPK, nitrogen, phosphorous and potassium

INTRODUCTION

The role of plants in the maintenance of good health has been reported (Burkill 1995; Moerman 1996). In Nigeria, these indigenous plants contain bioactive compounds that exhibit physiological activities against bacteria and other microorganisms and are also used as precursor for the synthesis of useful drugs (Sofowara 1993; Okwu 2001; Edeoga *et al.* 2003; Osuagwu *et al.* 2007; Osuagwu 2008). The antimicrobial activities of these plants and their products such as essential oils are well documented (El-Zaher *et al.* 2006; Ijeh *et al.* 2006; Mevy *et al.* 2007; Sahraoui *et al.* 2007; Vagionas *et al.* 2007; Nwinyi *et al*. 2008; Sengui *et al*. 2009; Pir-balouti *et al*. 2010). Thus these plants are therefore used in the treatment of many diseases such as rheumatism, diar-rhea, malaria, elephantiasis, cold obesity, dysentery, high blood pressure, malnutrition, gonorrhea and others (Gill 1992; Burkill 1995; Batram 1998; Edet *et al*. 2009; Aku-odor *et al*. 2010).

The biosynthesis of these bioactive plant chemicals is influenced by various agronomic and environmental factors. Fertilizer treatment is known to determine the concentration of these compounds in plants (Asami *et al.* 2003; Khalil *et al.* 2007; Osuagwu and Nwachukwu 2007; Saradhi *et al.* 2007; Rasmussen *et al.* 2008; Alizadeh *et al*. 2010; Osuagwu and Edeoga 2010; Osuagwu *et al*. 2010). Water stress (drought) is also reported to influence the concentration of these phytochemicals in plants (Zheng *et al.* 2006; Selmar 2008).

The antimicrobial activities of *Ocimum gratissimum* and *Gongronema latifolium* have been investigated and reported. The antimicrobial activities of *O. gratissimum* have been documented (Iwalokun *et al*. 2003; Ezekwesili *et al.* 2004; Ijeh *et al.* 2005; Tchoumbougnong *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 the work of Afolabi and Eleyinmi (2007) showed that water and methanol extracts of *G. latifolium*, significantly inhibited the growth of some pathogenic bacteria.

The objective of this research is to ascertain the implication of fertilizer treatment on the antimicrobial activities of the leaves of *O. gratissimum* and *G. latifolium*.

MATERIALS AND METHODS

Plant samples

The seeds of *O. gratissimum* were collected from a homestead garden in Amaogwu village Bende town, Bende Local Government Area of Abia state. The fresh and succulent stem cuttings of *G. latifolium* were obtained from the forest strip of the Forest 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 Department of Biological Sciences, 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 24 plastic buckets containing 8 kg of sterilized soil. The soil used for the research was analyzed to determine the physiochemical properties (**Table 1**).

Treatments were carried out in four replicates of each treatment. The inorganic fertilizer (NPK 15:15:15) used for the study was obtained from the store of the Abia State Ministry of Agriculture, Umuahia, Abia State. Five levels of fertilizer treatments 100,

Received: 23 July, 2009. Accepted: 2 December, 2010.

Original Research Paper

Table 1 Physiochemical properties of soil.

Particle size distribution	
Sand	70.90%
Silt	15.40%
Clay	13.70%
Texture	5 L
pH (H_2O)	5.01
Organic carbon (%)	0.75%
Organic matter (%)	1.29%
Available phosphorus (mg/Kg)	46.00
Total nitrogen (%)	0.08%
Exchangeable bases (mg/100 g)	
Ca^{++}	2.40
Mg^{++}	2.00
K^{+}	0.07
Na^{+}	0.23
Exchangeable acidity (ME/100 g)	1.20
Effective cation exchange capacity (ME/100 g)	5.90

200, 300, 400 and 500 kg/ha derived using the furrow slice method (Brady and Weil 1999), in four replicates were used. No fertilizer treatment served as control. Treatment occurred two months after seedling emergency. Harvesting of plants leaves for antimicrobial activity investigation was carried out one month after treatment.

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).

Fifty grams of the pondered samples was soaked in 200 ml of absolute ethanol and allowed to stand for 24 h. It was filtered using a Whatman No. 1 filter paper. The filtrate was evaporated to dryness over 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 the stock culture of the Microbiology laboratory, Federal Medical Centre, Umuahia, Abia State, Nigeria. Viability test of each isolate was carried out by resuscitating the organisms in buffered peptone broth and thereafter sub-cultured into nutrient agar medium and incubated at 37°C for 24 h.

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).

20 ml of the molten nutrient agar was seeded with 0.2 ml of broth culture of the test organisms in sterile Petri dishes. The Petri dishes were rotated slowly to ensure a uniform distribution of the organisms. They were left to solidify and in the dish cups of 8.0 mm diameter were made in the agar using a sterile Pasteur pipette. The Petri dishes were allowed to stand for about 30 minutes at room temperature to allow for proper diffusion of the extracts to take place. The plates were then incubated at 37°C for 24 h. The zone of inhibition in millimeter 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 design for the research was complete randomized design in four replicates of each treatment. Analysis of variance (ANOVA) was used to analyze the data and LSD at $P<0.05$ was used to determine the difference among treatments.

RESULTS AND DISCUSSION

Fertilizer treatment significantly affected the antimicrobial activity of the leaves of *O. gratissimum* and *G. latifolium* (**Table 2**).

There was significant increase in the ability of the leaves of *O. gratissimum* to inhibit the activity of *K. pneumoniae*, *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *S. faecalis*, *C. albicans* due to fertilizer treatment (**Table 2**). Fertilizer treatment also significantly increased the ability of the leaves of *G. latifolium* to inhibit the activity of *K. pneumoniae*, *E. coli*, *S. aurues*, *P. aeruginosa*, *S. typhi*, *S. faecalis* and *C. albicans* (**Table 2**).

The observed increased ability of the leaves of *O. gratissimum* and *G. latifolium* to inhibit the microbial activity of these pathogens as testified by the inhibition zones in response to fertilizer treatment might be related to the enhanced synthesis and accumulation of phytochemicals (alkaloids, phenols, saponins, steroids, tannins) and other plant chemicals substances by these plants a consequence of fertilizer treatment. This in turn increased the ability of the extracts of the leaves to inhibit the activity of these pathogens. Osuagwu and Nwachukwu (2007) and Osuagwu (2008) had observed that organic fertilizer application led to increased

Table 2 Effect of NPK fertilizer treatment of the antimicrobial activity of the leaves of *Ocimum gratissimum* and *Gongronema latifolium* on *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aurues*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger*.

Treatment	Control	100 kg/ha	200 kg/ha	300 kg/ha	400 kg/ha	500 kg/ha	LSD (<0.05)
	Zone of inhibition (mm)						
Ocimum gratissimum							
Klebsiella pneumoniae	20.00 ± 0.707 a	25.00 ± 0.913 b	27.00 ± 0.910 bc	29.00 ± 0.906 c	32.00 ± 1.472 d	34.00 ± 0.913 d	1.414
Escherichia coli	26.00 ± 0.913 a	28.00 ± 1.472 ab	30.00 ± 0.912 bc	32.00 ± 1.473 cd	34.00 ± 1.291 de	36.00 ± 1.472 e	1.810
Staphylococcus aurues	13.00 ± 0.913 a	21.00 ± 1.190 b	24.00 ± 1.502 bc	26.00 ± 1.472 cd	28.00 ± 1.290 cd	29.00 ± 1.472 d	1.863
Pseudomonas aeruginosa	16.00 ± 1.112 a	16.00 ± 1.472 ab	20.00 ± 1.470 bc	23.00 ± 0.913 cd	24.00 ± 1.472 d	26.00 ± 0.646 d	1.691
Salmonella typhi	17.00 ± 0.913 a	20.00 ± 1.472 ab	22.00 ± 0.912 bc	25.00 ± 1.472 cd	27.00 ± 0.913 d	28.00 ± 1.291 d	1.599
Streptococcus faecalis	15.00 ± 0.911 a	18.00 ± 1.472 b	21.00 ± 1.155 b	22.00 ± 1.323 bc	25.00 ± 0.913 cd	27.00 ± 1.472 d	1.740
Candida albicans	7.00 ± 0.913 a	12.00 ± 0.913 bc	14.00 ± 1.472 c	10.00 ± 1.472 ab	12.00 ± 0.913 bc	13.00 ± 0.912 bc	1.599
Aspergillus niger	0.00	0.00	0.00	0.00	0.00	0.00	-
Gongronema latifolium							
Klebsiella pneumoniae	13.00 ± 0.913 a	20.00 ± 0.913 b	24.00 ± 1.291 c	26.00 ± 0.913 cd	28.00 ± 0.913 d	32.00 ± 1.472 e	1.546
Escherichia coli	15.00 ± 0.913 a	15.00 ± 0.913 a	27.00 ± 0.913 c	30.00 ± 1.472 cd	32.00 ± 0.913 d	35.00 ± 1.472 e	1.740
Staphylococcus aurues	15.00 ± 0.913 a	23.00 ± 1.851 b	26.00 ± 1.704 bc	25.00 ± 1.472 bc	28.00 ± 1.264 cd	30.00 ± 0.935 d	1.732
Pseudomonas aeruginosa	10.00 ± 0.912 a	17.00 ± 1.470 b	21.00 ± 1.924 c	20.00 ± 1.573 bc	25.00 ± 0.913 d	25.00 ± 0.913 d	1.683
Salmonella typhi	12.00 ± 0.965 a	12.00 ± 0.965 a	26.00 ± 0.913 bc	22.00 ± 1.742 b	26.00 ± 1.290 bc	29.00 ± 1.471 c	1.764
Streptococcus faecalis	14.00 ± 1.473 a	22.00 ± 1.537 b	25.00 ± 1.174 c	22.00 ± 0.986 b	24.00 ± 1.975 b	26.00 ± 1.276 c	1.856
Candida albicans	5.00 ± 0.927 a	14.00 ± 0.913 b	16.00 ± 1.295 bc	16.00 ± 1.643 bc	16.00 ± 1.473 bc	19.00 ± 1.896 c	1.683
Aspergillus niger	0.00	0.00	0.00	0.00	0.00	0.00	-

concentration of flavonoid, phenol, saponin and tannin in the leaves of *Ocimum gratissimum.* Their reports tend to agree with that of Alizadeh *et al*. (2010), which showed that fertilizer treatment caused increased total phenolic content in *Satureja hortensis.* These bioactive substances are known to have antimicrobial properties (Edeoga *et al*. 2009). Enhanced production of phytochemicals, vitamins and other plant chemical substances by plants as a result of fertilizer treatment has been reported. Das *et al*. (2006) showed that fertilizer treatment caused significant increase in the saponin stevioside content of *Stevia rebaudiana*. The alkaloid content of the tissues of *Solidago virgaurea* was also reported to be increased by NPK fertilizer treatment (Kolodziej 2007). Increased flavonoid, phenol and tannin instigated by fertilizer treatment was documented (Zheng *et al*. 2006; Sengui *et al*. 2009). The essential oils content of *Tagetes minota* and *Baccharis trimers* was observed to be increased by fertilizer treatment (Silva *et al*. 2006; Omidbaigi *et al*. 2008). Osuagwu *et al*. (2010) also reported that fertilizer treatment affected the yield and chemical constituents of the essential oil from the leaves of *Ocimum gratissimum*. Furthermore, Mozaffar (1994) and Polat (2008) reported that fertilizer treatment significantly increased the ascorbic acid, thiamine and niacin content of some plants. The elevation of the concentration of these bioactive compounds as a result of fertilizer treatment invariably increased the potency of these medicinal plants. These phytochemicals are known to exhibit physiological activities against bacteria and other microorganisms (Sofowora 1993; Okwu 2001).

The antimicrobial activities of *O. gratissimum* and *G. latifolium* have earlier on been investigated. The antimicrobial activity of *O. gratissimum* was widely reported (Iwalokun *et al*. 2003; Holetz *et al*. 2003; Ezekwesili *et al*. 2004; Nakamura *et al*. 2004; Adebolu and Oladimeji 2005; Matasyoh *et al*. 2007; Nweze and Eze 2009; Nwinyi *et al*. 2009; Oboh *et al*. 2009). The use of *O. gratissimum* as a herbal medicine for the treatment of diseases such as upper respiratory tract infection, diarrhea, pile, cough, fever, pneumonia, surface wound, gonorrhea, worm infestation and stomach aches has been documented (Gill 1992; Burkill 1995; Okeke 1998; Iwalokun *et al.* 2003; Nangia-Makker *et al.* 2007; Prabhu *et al*. 2009). The leaf extracts are used to reduce blood glucose levels (Owoyele *et al.* 2005; Mohammed *et al.* 2007). Its role in blood coagulation and renal function is reported (Edemeka and Ogwu 2001; Anigbogu and Uzoaga 2006). The leaves of *O. gratissimum* are used to prepare soups and porridge for women after delivery among the Igbo's of Nigeria (Ijeh *et al.* 2004). The leaves are also used as spices for preparation of food (Burkill 1995; Ijeh *et al*. 2004).

The antimicrobial activity of the leaf extracts of *G. latifolium* has been shown by Afolabi and Eleyinmi (2007). It is used in traditional medicine for the treatment of cough, loss of appetite, diabetes and improved liver function (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). Asthmatic patients usually chew fresh leaves of *G. latifolium* to relieve wheezing (Nwosu and Malize 2006). Leaves are used as spices or condiment in the diet of nursing mothers and are used raw in 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 brewing of beer (Nwosu and Malizie 2006).

The leaf extracts of *O. gratissimum* and *G. latifolium* had no antimicrobial effect on *Aspergillus niger*. The leaves of both treated and untreated plants did not inhibit the microbial activity of *A. niger*. This indicates that *A. niger* is resistant to the chemicals contained in the leaves of the plants.

The extracts of the leaves of *O. gratissimum* and *G. latifolium* had more inhibitory effect on the microbial activity of bacteria, when compared with effect on fungi (**Table 2**). The above observation relates to the reactions of different organisms to similar and different environmental conditions.

Furthermore, generally increasing the level of fertilizer treatment led to corresponding increase in the ability of the leaves of the plants to inhibit the microbial activity of the pathogens, which in turn the functions of the quantity of phytochemicals they contain. Wiesler (1997) observed that the impact of nitrogen fertilizer on metabolism of plants depends on factors such as its concentration in the soil and chemical form.

The importance of the finding from this research is that the application of NPK fertilizer at the appropriate levels will enhance the antimicrobial activity of the leaves of *O. gratissimum* and *G. latifolium*, and thus increase their value and efficacy as medicinal plants.

REFERENCES

Adebolu TT, Oladimeji SA (2005) Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhea causing bacteria in South Western Nigeria. *African Journal of Biotechnology* **4 (7)**, 682-684

Afolabi F, Eleyinmi A (2007) Chemical composition and antibacterial activity of *Gongronema latifolium*. *Journal Zheijiang University of Sciences B* **8 (5)**, 352-358

Akuodor GC, Idris-Usman MS, Mbah CC, Megwas UA, Akpan JL, Ugwu TC, Okoroafor DO, Osunkwo UA (2010) Studies on anti-ulcer, analgestic and anti pyretic properties of the ethanolic leaf extract of *Gongronema latifolium* in rodents. *African Journal of Biotechnology* **9 (5)**, 2316-2321

Alizadeh A, Khoshkhui M, Jauidnia K, Firuzi O, Tafazoli E, Khalighi A (2010) Effect of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (Lamiaceae) cultivated in Iran. *Journal of Medicinal Plants Research* **4 (1)**, 33-40

Anigbogu CM, Uzoaga KI (2006) The effects of *Ocimum gratissimum* leaf extract on cardiovascular and renal function in rats. *Nigerian Quarterly Journal of Hospital Medicine* **16 (2)**, 60-66

Bartram J (1998) *Encyclopedia of Herbal Medicine*, Robinson, London, 490 pp

Brady NC, Weil RR (1999) *The Nature and Properties of Soils* (12th Edn), Prentice Hall, New Jersey, 881 pp

Burkill HM (1995) *The Useful Plants of West Tropical Africa* (Vol 3, families J–L), Royal Botanic Garden, Kew, 605 pp

Burkill IH (1985) *The Useful Plants of West Tropical Africa* (Vol X, families A–D), Royal Botanical Garden. Kew, 691 pp

Das K, Danga R, Shivananda TN (2006) Effect of major nutrients on biomass production and stevioside content of *Stevia rebaundiana* on pot culture study. *Biomedical* **1 (1)**, 22-25

Ebi GC, Ofoefule SI (1997) Investigation into the folkloric antimicrobial activities of *Landolphia owerriance*. *Phytotheraptic Research* **11**, 147-151

Edemeka DBU, Ogwu AS (2001). Blood coagulation activities of the leaf extracts of *Ocimum gratissimum* plant in man. *Journal of Herbs, Spices and Medicinal Plants* **7 (4)**, 9-14

Edeoga HO, Okwu DE, Mbaebie BO (2003) Mineral and nutritive value of some Nigerian medicinal plants. *Journal of Medicinal and Aromatic Plant Sciences* **25**, 689-694

Edeoga HO, Osuagwu GGE, Omosun G, Mbaebie BO, Onwuka AS (2009) Chemical characters and utility of some Rubiaceous medicinal plants from Nigeria. *Recent Progress in Medicinal Plants Volume 23 Phytopharmacology and Therapeutic Values* **5**, 81-89

Edet EE, Akpanabiatu MI, Eno AE, Umoh IB, Itam EH (2009) Effect of *Gongronema latifolium* crude extract on some cardiac enzymes of alloxan-induced diabetic rats. *African Journal of Biochemistry Research* **3 (11)**, 366-369

El-Zaher FHA, Fayez M, El-Maksoud HKA, Hosny I (2006) Antimicrobial activity of some Egyptian medicinal and aromatic plant waste extract. *Bulletin of the National Research Centre (Cairo)* **31 (1)**, 1-20

Ezekwesili CN, Obiora KA, Ugwu OP (2004) Evaluation of anti-diarrhoeal property of crude aqueous extracts of *Ocimum gratissimum* L. (Labiatae) in rats. *Biokemistri* **16 (2)**, 122- 131

Gills LS (1992) *Ethnomedicinal Uses of Plants in Nigeria*, University of Benin Press, Benin, Nigeria, 124 pp

Hoft M, Verpoorte R, Beck E (1996) Growth and alkaloid contents in leaves of *Tabernaemontana pachysiphon* Staff (Apocynaceae) as influenced by light intensity, water and nutrient supply. *Oecologia* **107 (2)**, 160-169

Holetz FB, Ueda-Nakamura Ficho BPD, Cortez DAG, Morgado-Diaz JA, Nakamura CV (2003) Effect of essential oil of *Ocimum gratissimum* on the hypanosomatid, *Herpetomanas sameulpesson*. *Acta Protozoologica* **42**, 269-276

Ijeh II, Njoku OU, Ekenze EC (2004) Medicinal evaluation of extracts of *Xylopia aethipica* and *Ocimum gratissimum*. *Journal of Medicinal and Aromatic Plant Sciences* **26**, 41-49

Ijeh II, Omodamiro OD, Nwanna IJ (2005) Antimicrobial effects of aqueous and ethanolic fractions of two spices, *Ocimum gratissimum* and *Xylopia aethiopica*. *African Journal of Biotechnology* **4 (9)**, 953-956

Iwalokun BA, Ghenle GO, Adewole TA, Akinsinde KA (2003) Shigellocidal properties of the Nigerian medicinal plants: *Ociumum gratissimum*, *Terminalia aicennaoides* and *Monmerdica balsamma*. *Journal of Health, Population and Nutrition* **19 (4)**, 331-335

Khalil MY, Moustafa AA, Naguib MY (2007) Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition. *World Journal of Agricultural Science* **3 (4)**, 451-457

Kolodziej B (2007) The effect of NPK fertilization on golden rod (*Solidago virgaurea,* L. subsp *Virgauera*) yield and quality parameters. *Herba Polonica* **53 (3)**, 129-134

Matasyoh IG, Matasyoh JC, Wachira FN, Kinyua MG, Muigal AW, Muklama TK (2007) Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. grown in Eastern Kenya. *African Journal of Biotechnology* **6 (6)**, 760-765

Mbata TI, Salkia A (2007) Antibacterial activity of essential oil from *Ocimum gratissimum* on *Listeria monocytogenes*. *Internet Journal of Food Safety* **5 (7)**, 15-19

Mevy JP, Bessiere JM, Dherbomez M, Millogo J, Viano J (2007) Chemical composition and some biological activities of the volatile oils of a chemotype of *Lippia chevalieri* Moldenke. *Food Chemistry* **101 (2)**, 682-685

Moerman DE (1996) An analysis of the food plants of nature. *North America Journal of Ethnopharmacology* **52**, 1-22

Mohammed A, Tanko Y, Okasha MA, Magaji RA, Yaro H (2007) Effects of aqueous leaves extract of *Ocimum gratissimum* on blood glucose levels of streptozocin-induced diabetic Wistar rats. *African Journal of Biotechnology* **6 (18/ 19)**, 2087-2090

Morebise O, Fafunso MA, Makinde JM, Olajide OA, Awe CO (2002) Anti-inflammatory property of the leaves of *Gongronema latifolium Phytotherapeutic Research* **16**, 57-67

Mozzafar A (1994) Enrichment of some B – vitamins in plants with application of organic fertilizers. *Plant and Soil* **167 (2)**, 305-3111

Nakamura CV, Ishida K, Facein LC, Filho BP, Cortez DA Rozental S, de Souza W, Ueda-Nakamura T (2004) *In vitro* activity of essential oils from *Ocimum gratissimum* L. against four *Candida* species. *Microbiological Research* **155 (7)**, 579-586

Nwanjo HU, Alumanah EO (2006) Effect of aqueous extract of *Gongronema latifolium* on some indices of liver function in rats. *Global Journal of Medical Sciences* **5 (1)**, 17-20

Nweze EI, Eze EE (2009) Justification for the use of *Ocimum gratissimum* L in herbal medicine and its interaction with disc antibiotics. *Biomedical Central Complementary and Alternative Medicine* **9**, 37

Nwinyi OC, Chinedu NS, Ajani OO, Ikpo CO, Ogunniran UA (2009) Anti bacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *African Journal of Food Sciences* **3 (3)**, 77-81

Nwosu MO, Malize N (2006) An anatomic-systematic study of medicinal plants of Nigeria (11). *Gongronema latifolium* Benth (Asclepladaceae). *Journal of Economic and Taxonomic Botany* **30 (2)**, 5-10

Oboh FOJ, Masodje HI, Enabulele SA (2009) Nutritional and antimicrobial properties of *Ocimum gratissimum* leaves. *Journal of Biological Sciences* **9 (4)**, 377-380

Okafor JC, Okolo HC, Ejiofor MAN (1996) Strategies for enhancements of utilization potential of edible wood forest species of southern-eastern Nigeria. In: *The Biodiversity of African Plants*, Kluwer Academic Press, The Netherlands, pp 686-695

Okeke CU, Elekwa I (2006) Comparative hypoglycemic effects of three Nigerian vegetable spices, *Gongronema latifolium* Benth, *Allium sativum* Linn and *Ocimum gratissimum* L. Alloxan-induced diabetic rats. *Nigerian Journal of Botany* **19 (1)**, 138-146

Okeke EC (1998) The use and chemical content of some indigenous Nigerian species. *Journal of Herbs, Spices and Medicinal Plants* **5 (4)**, 51-63

Okwu DE (2001) Evaluation of the chemical composition of indigenous species and flavouring agents. *Global Journal of Pure and Applied Sciences* **7 (3)**, 455-459

Omidbaigi R, Dadman B, Fattahi F (2008) Influence of nitrogen fertilizer on the herb yield, essential oil content and composition of *Tagetes minota* L. *Journal of Essential Oil-Bearing Plants* **11 (1)**, 45-52

Oshinubi RA, Emeke PM, Awodele O (2006) The effect of ethanolic stem extracts of *Gongronema latifolium* on blood glucose of normal and alloxan induced diabetic rabbits. *Nigerian Journal of Health and Biomedical Sciences* **5 (2)**, 39-44

Osuagwu GGE (2008) The effect of rate of application of poultry manure on the phenol, flavonoid and steroid potential of the leaves of *Ocimum gratissimum. Journal of Sustainable Agriculture Environment* **10 (2)**, 106-111

Osuagwu GGE, Edeoga HO (2010) The effect of NPK inorganic fertilizer application on the concentration of mineral and vitamin in the leaves of *Gongronema latifolium* (Benth). *Recent Progress in Medicinal Plants Volume 30*; *Drug Plants* **4**, 167-177

Osuagwu GGE, Edeoga HO, Osuagwu AN (2010) The effects of inorganic fertilizer application and water stress on the essential oils of the leaves of *Ocimum gratissimum* (L). *Nigerian Journal of Botany* **23 (1)**, 122-131

Osuagwu GGE, Nwachukwu CM (2007) Effect of organic fertilizer application on the alkaloids, saponin, tannins and cyanogenic glycosides constituents of *Ocimum gratissimum. Environment and Ecology* **255 (4A)**, 1270-1275

Osuagwu GGE, Okwulehie IC, Emenike JO (2007) Phytochemical and mineral content of the leaves of four Nigerian *Pterocarpus* species. *International Journal of Molecular Medicine and Advance Sciences* **3 (1)**, 6-11

Owoyele VB, Adeyemi FM, Soladoye AO (2005) Effect of aqueous leaves extracts of *Ocimum gratissimum* (sweet basil) on alloxan-induced diabetic rats. *Pharmacognosy Magazine* **1 (2)**, 62-64

Pirabalouti AG, Malekpor F, Enteshari S, Yousef M, Momfaz H, Hammed B (2010) Antibacterial activity of some folklore medicinal plants used by Bakhtiari Tribal in South *West Iran International Journal of Biology* **2 (2)**, 1-9

Polat E, Demir H, Onys AN (2008) Comparison of some yield and quality criteria in organically and conventionally grown lettuce. *African Journal of Biotechnology* **7 (9)** 1235- 1239

Prabhu KS, Lobo R, Shirwaikar AA, Shirwaikar A (2009) *Ocimum gratissimum*: A review of its chemical, pharmacological and ethnomedicinal properties. *The Open Complementary Medicine Journal* **1**, 1-15

Rasmussen S, Parsons AJ, Fraser K, Xue H, Newman JA (2008) Metabolic profiles of *Lolium perenneare* as differentially affected by nitrogen supply, carbohydrate content and fungal endophy to infection. *Plant Physiology* **146** 1440-1453

Sahraoui M, Hellal A, Boutekedjiret C, Bentahar F, Bessiere JM (2007) Antimicrobial activities of essential oils of some Algerian aromatic plants. *Therapeutica* **1 (2)**, 83-90

Saradhi VSP, Salma K, Shivananda BY, Kumar TV, Shivananda TN (2007) Effect of NPK fertilizers on chemical constituents of *Aloe vera* leaves. *Journal of Natural Remedies* **7 (2)**, 258-262

Selmar D (2008) Potential of salt and drought stress to increase pharmaceutical significant of secondary compounds in plants. *Landbauforschong, Volume Kendrode* **58 (112)**, 139-144

Sengui M, Vildiz H, Gungor N, Cetin B, Zeynep E, Ercisli S (2009) Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pakistan Journal of Pharmaceutical Sciences* **12 (1)**, 102-106

Silva FY, Pinto JE, Cardoso MG, Sales JF, Mol DJS, Divino SP (2006) Influence of manure and fertilizer on *Baccharis trimers* (less) d c. growth essential oil yield. *Journal of Herbs, Spices and Medicinal Plants* **12 (1/2)**, 1-11

Sofowara A (1993) *Medicinal Plants and Traditional Medicine in Africa*, Spectrum Books Limited, Ibadan, Nigeria, 289 pp

Tchoumbougnang PH, Zollo A, Dongne E, Mekonnen Y (2005) *In vivo* antimalarial activity of essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* L. on mice infected with *Plasmodium berghei*. *Planta Medica* **71**, 20-23

Ugochukwu NH, Babady NE, Coboume M, Gasset SR (2003) The effect of *Gongronema latifolium* extracts on serum lipids profile and oxidative stress in hepatocytes of diabetic rats. *Journal of Biological Sciences* **28 (1)**, 1-5

Vagionas K, Graikou K, Ngassapa O, Rongyoro D, Chinou I (2007) Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania. *Food Chemistry* **103 (2)**, 319-324

Wiesler F (1997) Agronomical and physiological aspects of ammonium and nitrate nutrition in plants. *Zeitschrift für Pflanzenernahring und Bondenkunde* **160**, 227-238

Zheng Y, Dixon M, Saxena PK (2006) Growing environment and nutrient availability affect the content of some phenolic compounds in *Echinacea purpurea* and *Echinacea angustifolia*. *Planta Medica* **72**, 1407-1414