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Nutritional and Antimicrobial Properties of *Ocimum gratissimum* Leaves

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Abstract: Fresh green *Ocimum gratissimum* leaves were analysed for protein content, moisture, ash, minerals and antimicrobial activity. The fresh leaves had a moisture content of 81.35%, a protein content of 1.21% and an ash content of 0.57%. On a fresh weight basis mineral content was as follows: phosphorus 52.4, selenium 0.007, iron 7.9 and zinc 1.5 $\mu\text{g g}^{-1}$. An aqueous extract of the leaves inhibited the growth of the gram positive bacterium *Staphylococcus aureus* and the gram negative bacterium *Escherichia coli*. The nutritional implications of the results are discussed.

Key words: *Ocimum gratissimum* L., moisture, protein, ash, minerals, antimicrobial, *Escherichia coli*, *Staphylococcus aureus*

INTRODUCTION

Leafy vegetables are a good source of dietary fibre, carotenoids, vitamin C, folate, phytochemicals and certain minerals, but have low concentrations of protein, digestible carbohydrates and lipids (Wills *et al.*, 1998). They are easy to cultivate and provide an inexpensive means of combating vitamin and mineral deficiency in less developed regions of the world. *Ocimum gratissimum* is found throughout the tropics and subtropics, both wild and cultivated. Its greatest variability occurs in tropical Africa, where it probably has its origin and India (Osuji *et al.*, 1995). The leaves of the plant, which are highly appreciated for their pleasant aroma are used as seasoning and are eaten as a vegetable. The plant has interesting medicinal properties (Gill, 1992). While, its essential oil have been extensively investigated (Simon *et al.*, 1990), little is known about its nutritional composition and antimicrobial activity. In the present study, the moisture, protein, ash and mineral (phosphorus, selenium, iron and zinc) content of *O. gratissimum* leaves and the antimicrobial effect of their aqueous extract on the bacteria *Escherichia coli* and *Staphylococcus aureus* were determined. The nutritional and food processing implications of the results are discussed.

MATERIALS AND METHODS

This study was conducted in Benin City, Nigeria in the months of May and June 2007.

Materials

Vegetables: Leaves of *O. gratissimum* were purchased from markets in Benin City. They were dark green in colour and freshly cut.

Microorganisms: *Escherichia coli* (ATCC 35218) and *Staphylococcus aureus* (N 315 methicillin resistant/gram + ve) were obtained respectively from the Veterinary Research Institute of Nigeria, Vom, Plateau State and the Nigerian Institute of Oceanography and Marine Research, Victoria Island, Lagos.

Reagents: All reagents were analytical grade.

Analytical methods

Moisture: Shredded fresh vegetable (10 g) was dried in a thermostatically controlled ventilated oven at 105°C until constant weight was obtained. The loss in weight was recorded as moisture content (AOAC, 1984).

Sample preparation for protein, ash and mineral analysis: Leaves were cut into tiny pieces and dried in a ventilated oven at 60°C for 5 days to constant weight. The dried vegetable was ground into powder and stored in airtight bottles for analysis.

Crude protein: Dried and pulverised leaf (0.2 g) was digested in 2 mL concentrated H_2SO_4 in the presence of selenium catalyst, until a clear digest was obtained

(AOAC, 1984). The nitrogen content of diluted digest was determined colourimetrically at 630 nm according to Charlot (1964). Protein was calculated as: nitrogen content \times 6.25.

Ash: For the determination of ash content, dried pulverised vegetable was ashed at 550°C in a muffle furnace.

Minerals: Minerals were obtained by ashing 2.0 g dried and ground sample in a muffle furnace at 550°C. The ash was dissolved in 10 mL, 20% nitric acid and filtered through a dry 9 cm acid washed Whatman No. 541 filter paper into a 100 mL volumetric flask. The filtrate was made up to the mark with deionised water and the resulting solution was used for the analysis of phosphorus, zinc and iron. Phosphorus was determined colourimetrically according to Charlot (1964). To mineral solution (10 mL) in a 50 mL volumetric flask was added 0.2 mL of 0.5% para-nitrophenol indicator solution. To this was added an ammonia solution (6 N) dropwise, with gentle shaking until the appearance of a yellow colour. Nitric acid (1 N) was then added in the same manner until the solution turned colourless. Finally, ammonium molybdate/ammonium vanadate mixed reagent (5 mL) was added. The solution was made up to 50 mL with distilled water; the flask was stoppered and its contents well mixed. The flask was allowed to stand for 30 min and the absorbance of the solution measured at 400 nm. Phosphorus content of the solution was read off a calibration graph prepared using potassium dihydrogen phosphate as standard. Zinc and iron were determined using an atomic absorption spectrophotometer (Buck Scientific VGP 210) at 630 nm (Okalebo, 1985). Selenium was determined titrimetrically (Charlot and Benzier, 1957). To an aqueous, extract of the leaves were added 5 mL starch solution and 1 g potassium iodide. The mixture was stirred, allowed to stand for 15 sec and titrated with 0.05 N sodium thiosulphate solution. The end point was marked by a change from a dirty or turbid solution to a transparent red colour. Analysis were done in duplicate.

Antimicrobial activity

Preparation of crude water extract of leaves: Aqueous extract of *O. gratissimum* leaves was prepared by blending 10 g fresh leaves in 100 mL peptone water (15 g peptone in 1 L water). The water extract was filtered through Whatman No. 1 filter paper and tested for antimicrobial activity.

Preparation of crude antibiotic discs: Sterile Whatman No. 1 paper was punched into 5 mm diameter disc sizes. The Whatman discs were placed in Mac Cartney bottles

and sterilised in an autoclave at 120°C for 15 min. The bottle was transferred into a hot air oven at 60°C to dry for 30 min. An aqueous extract of the leaves (1.0 mL) was transferred into a sterile Bijou bottle containing sterile discs. The sterile crude discs were allowed to soak in extract for 6 h for proper absorption, after which they were removed and allowed to dry (Cheesebrough, 2000).

Antimicrobial assay of extract: Aqueous extract of *O. gratissimum* leaves was screened *in vitro* for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Nutrient agar (7 g) was added to 250 mL distilled water in a flask. This was stirred and autoclaved at 115°C and then cooled to 50°C. A portion of the medium (20 mL) was poured into a sterile petri dish and allowed to solidify. The sterility of the medium was confirmed by allowing it to stay for 8 h and observing no contamination. An isolate colony of each test organism was subcultured on nutrient broth and incubated at 37°C for 8 h. This was then spread on the entire plate medium to ensure uniform growth. The crude extract discs were incubated immediately for 24 h at 37°C (Ogbulie *et al.*, 1998). Antimicrobial assays were carried out in quadruplicate. Zones of inhibition were observed using a hand lens for proper magnification and the zones measured.

RESULTS AND DISCUSSION

Ocimum gratissimum leaves were purchased freshly cut and were dark green in colour. Their moisture, protein and ash content are shown in Table 1. Included for comparisons are published data for some locally available vegetables, cassava leaves, cabbage, cowpea leaves and sweet potato leaves.

Water was the dominant component, constituting 81.35% of *O. gratissimum* leaves and 79-85% of the others. Protein constituted 1.21% of fresh *O. gratissimum* leaves, lower than the published values for fresh cassava leaves (6.0%), cabbage (1.4%), fresh cowpea leaves (4.1%) and sweet potato (4.6%). Ash constituted 0.57% of fresh *O. gratissimum* leaves. Overall, foods with a high water and dietary fibre content provide a low energy density contribution to the meal and create a feeling of satiety. The leaves had a low protein content.

Generally, fresh leafy vegetables have low protein content. This protein is mostly in the form of enzymes, rather than acting as a storage pool, as in grains and nuts (Wills *et al.*, 1998). The leaves of cassava, cowpea and sweet potato and cabbage appear to be better sources of dietary protein than *O. gratissimum* leaves. Thus fresh *O. gratissimum* leaves may not be an important source of

dietary protein. Ash content was low, indicating a low mineral content. The concentration in *O. gratissimum* leaves, of the macromineral phosphorus and the trace minerals, selenium, iron and zinc were determined. Table 2 shows the mineral content of the leaves. Included for comparison are the published values for fresh cassava and cowpea leaves and cabbage and the recommended daily allowances for the minerals.

Phosphorus constituted $52.4 \mu\text{g g}^{-1}$ (i.e., 0.4% of RDA was present in 100 g of fresh leaves), zinc $1.5 \mu\text{g g}^{-1}$ (i.e., 1.0% of RDA in 100 g fresh leaves) and iron $7.9 \mu\text{g g}^{-1}$ of fresh *O. gratissimum* leaves. These were lower than published values for cabbage and cowpea leaves except for the iron content of cabbage, which was lower. The concentration of selenium in leaves was $0.007 \mu\text{g g}^{-1}$ (1.3% of RDA in 100 g of fresh leaves). The iron contained in 100 g fresh *O. gratissimum* leaves represents about 5-8% of its RDA. The contributions of the other minerals studied to their total dietary requirements appear to be small. Thus *O. gratissimum* leaves may be a minor dietary source of these minerals. It is important to note however, that the nutritional value of vegetables depends not only on the concentration of nutrients in the produce, but also on the amount consumed in the diet. Also, since vegetables are usually eaten in combination with other dietary components,

some of which may be better sources of the minerals under consideration, they could be of value in supplementing the minerals available from these better sources. The effect of an aqueous extract of *Ocimum gratissimum* on the gram positive bacterium *Staphylococcus aureus* and the gram negative bacterium *Escherichia coli* was shown in Table 3.

Both showed sensitivity to the extract, giving zones of inhibition of 1.0 and 1.2 cm, respectively. Thus the aqueous extract of *Ocimum gratissimum* exhibited broad spectrum antimicrobial activity by inhibiting the growth of gram +ve and gram -ve organisms. Leafy vegetables are high moisture, low acid produce, which support the growth of a wide range of microorganisms. Thus great care is needed when processing them, in order to minimize poisoning, especially those, for example *S. aureus* and *E. coli*, which produce heat stable toxins that may not be destroyed by heat treatment such as cooking (James and Kuipers, 2003; Fellows and Axtell, 2001; Schmidt, 1983). *Staphylococcus aureus* and *Escherichia coli* are common food poisoning bacteria. The antimicrobial activity of *O. gratissimum* leaves could inhibit the growth of these bacteria on the vegetable itself and (by extraction during cooking, if the antimicrobial principle is heat stable) in food, e.g., sauces and stews in which it is an ingredient, thus protecting the consumer from their harmful effect.

CONCLUSIONS

Ocimum gratissimum leaves contained mainly water. Protein and ash were minor components. The elements studied in this study occur in appreciable concentrations in *Ocimum gratissimum* leaves. However, except for its iron content, which could be a major contributor to its RDA, the potential contribution of the other elements studied-phosphorus, selenium and zinc, to their RDA appears to be minor. The cold aqueous extract of the leaves inhibited the growth of *S. aureus* and *E. coli*.

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Table 1: Moisture, protein and ash content (% weight) of fresh *Ocimum gratissimum*, cassava, cowpea and sweet potato leaves and cabbage

Variables	<i>O. gratissimum</i> ^a	Cassava ^b	Cabbage ^b	Cowpea ^c	Sweet potato ^b
Moisture	81.35	80.0	79.0	89.8	83.0
Protein	1.21	6.0	1.4	4.1	4.6
Ash	0.57	-	-	-	-
Colour	Dark green	Dark green	Light green	Dark green	Dark green

^aThis study. ^bVan Gastel and van den Wijngaart (1997). ^cMadamba *et al.* (2006)

Table 2: Mineral content ($\mu\text{g g}^{-1}$ of *Ocimum gratissimum*, cabbage and cowpea leaves and recommended daily allowances (RDA) in mg for minerals

Minerals	<i>O. gratissimum</i> ^a	Cabbage ^b	Cowpea ^c	RDA ^b (% of RDA ^a)
Phosphorus	52.4	540.0	90.0	1300 (0.4)
Selenium	7.0×10^{-3}	-	-	0.055 ^d (1.3)
Iron	7.9	6.0	19.0	10-15 (5-8)
Zinc	1.5	3.0	3.0	15 (1.0)

^aThis study. Adapted from Fellows and Axtell (2001). Adapted from Madamba *et al.* (2006). ^bWardlaw and Kessel (2002). ^cPercentage of the RDA for mineral, which is present in 100 g vegetable

Table 3: Zones of inhibition (cm) of bacteria by aqueous extract of *Ocimum gratissimum* leaves

Microorganism	Zone of inhibition
<i>Staphylococcus aureus</i>	1.0
<i>Escherichia coli</i>	1.2

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