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Antioxidant and Antimicrobial Properties of Ethanolic Extract of *Ocimum gratissimum* Leaves

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Abstract: In an attempt to explain the scientific basis for the medicinal and nutritional benefits of the leafy vegetable, the phytochemical constituent, antioxidant and antimicrobial activity were assessed. In this study, dried leaves of *Ocimum gratissimum* were extracted with rectified spirit and the extracts were subsequently analyzed for their phytochemical constituent, antioxidant property as typified by its total phenol content, free radical scavenging ability and reducing power, as well as the ability of the extracts to inhibit the growth of some clinically isolated *Enterobacterioceae* and some fungi. The result of the study revealed that *Ocimum gratissimum* extract contains tannin, saponin, anthraquinone, alkaloid and glycosides, the total phenolic content of the extract was 3.6 g/100 g, while the reducing power and free radical scavenging ability were 2.4 OD_{700} and 51.2%, respectively. 1.0 mg mL^{-1} of the extract inhibited the growth of the following bacteria: *Proteus* sp. (3.4 mm), *Pseudomonads aeruginosa* (5.0 mm), *Shigella dysentria* (29.0 mm) and *Staphylococcus aureus* (18.0 mm) and fungi [*Saccharomyces cerevisae* (9.0 mm) and *Candida albicans* (13.0 mm)]. Furthermore, the inhibition of both the fungi and bacteria were found to be dose-dependent. However, same concentration of the extract will not inhibit the growth of the following bacteria: *Klebsiella* sp., *Escherichia coli* and some fungi namely; *Penicillium* sp., *Aspergillus fumigatus*, *Fusarium solani* and *Aspergillus flavus*. It could therefore be concluded that part of the reasons for the use of *Ocimum gratissimum* leaves in folk medicine against gastrointestinal disorders and haemorrhoids could be as result of its antioxidant and antimicrobial property which is hinged on the array of pharmacological active phytochemicals present in the vegetable leaves.

Key words: *Ocimum gratissimum*, phytochemicals, antioxidants, antimicrobial

Introduction

Ocimum gratissimum L. (Labiatae) is widely distributed in tropical and warm temperature regions. The plant is commonly used in folk medicine to treat different diseases e.g. upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia and also as a treatment for cough, fever and conjunctivitis (Cornea,1932; Onajobi, 1986). Previous studies showed that the Essential Oils (EO) of four *Ocimum* species grown in Rwanda, i.e., *O. canum*, *O. gratissimum*, *O. trichodon* and *O. urticifolium*, display antimicrobial activity (Janssen *et al*., 1989). It has been reported that the volatile oil of this plant contains mostly phenols, particularly thymol (Oliver, 1960; Sainsbury and Sofowora, 1971) and that these are probably responsible for its reported antimicrobial action.

The oil extracted from this plant contains the following compounds: 1,8 cineol, eugenol, methyl-eugenol, thymol, p-cimene, cis-ocimene and cis-caryophyllene and different concentrations of the oil inhibited the growth of *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella enteritidis*, *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Fusarium*

verticillioides (Ntezurubanza *et al*., 1987; Jedickova *et al*., 1992; Ndounga and Quamba, 1997; Nakamura *et al*., 1999; Fadohan *et al*., 2004). While the aqueous extract of the leaf had been reported to have antimicrobial (Iwalokun *et al*., 2003) and antidiarrhoea activity, the extract inhibited castor oil-induced diarrhoea in rats as judged by decrease in the number of wet faeces in the extract-treated rats. In addition, the extract inhibited the propulsive movement of intestinal contents. On the isolated ileum of guinea-pig, the extract showed no direct action; however, it reduced the responses of the guinea-pig ileum to acetylcholine, nicotine and histamine, phytochemical tests revealed that the main constituents of the aqueous extracts are tannins, steroids, triterpenoid and carbohydrates (El-Said *et al*., 1969).

Since many degenerative human diseases have been recognized as being a consequence of free radical damage, the most practical way to fight against degenerative disease is to improve body antioxidant status, which could achieved by higher consumption of vegetables and fruits (Oboh and Akindahunsi, 2004; Oboh, 2005). The protective action of vegetables has been attributed to the presence of antioxidants, especially antioxidant vitamins including ascorbic acid, α-tocopherol and β-carotene. However, numerous studies have conclusively shown that the majority of the antioxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin rather than from vitamins C, E and β-carotene (Chu *et al*., 2002). The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Alia *et al*., 2003).

In Nigeria, *Ocimum gratissimum* is found in the Savannah and Coastal areas, the plant is used in the treatment of epilepsy, high fever and diarrhea, whilst in Savannah areas decoctions of the leaves are used to treat mental illness (Onajobi, 1986). In the Southern part of Nigeria, the Yoruba speaking tribe calls the plant "Efinrin", the Igbos calls it "Ahuji", while the Hausas call it "Daidoya" (Onajobi, 1986). It is also popularly used as a condiment in the preparation of local soup in Nigeria (Oboh and Akindahunsi, 2004; Oboh, 2005) or as infusion prepared in aqueous (boiled in water) or ethanol medium locally referred to as "Ogogoro" in folk medicine preparation. While a lot of information abound on the phytochemical constituent and biological activity of the essential oil and aqueous extract of *Ocimum gratissimum* leaf, there is a dearth of information with regard to the phytochemical constituent, antimicrobial and antioxidant activity of ethanolic extract of *Ocimum gratissiumum* leaf, this study therefore sought to assess the antioxidant properties of the ethanolic extracts of the vegetable as typified by its free radical scavenging ability and reducing power and to determine the ability of the extracts to inhibit some of the commonly encountered *Enterobacteriocea* and some fungi in Nigeria in an attempt to provide explanation for its nutritional and medicinal benefits.

Materials and Methods

Materials

Ocimum gratissimum leaves were collected from a local market in Akure, Nigeria, while the authentication was done at the department of Crop Production, Federal University of Technology, Akure. The water used was glass-distilled, while the chemicals were analytical grades. The bacteria tested (*Proteus* sp., *Pseudomonads aeruginosa*, *Shigella dysentria, E.coli, Klebsilla* sp. and *Staphylococcus aureus*) were collected from Ondo State Specialist Hospital, Akure Nigeria, while the fungi (*Saccharomyces cerevisae, Aspergillus fumigatus, Fusarium solani, Aspergillus flavus* and *Candida albicans*) were collected from Microbiology Department, Federal University of Technology Akure, Nigeria.

Preparation of Extracts

Fresh leaves of *Ocimum gratissimum* were homogenized with 95% ethanol using a PowerGen 1800 D homogenizer and the mixture was filtered with Whatman No.1 filter paper. And the filterate was concentrated to 1/10 of its original volume at 40°C and freeze-dried.

Phytochemical Screening

The ethanolic extracts of *Ocimum gratissimum* leaf was screened for the presence of alkaloids, tannins, saponnin, anthraquinone and cardiac glucosides using the method of Farnsworth *et al*. (1974).

Antioxidant Property

Total Phenol Content

The total phenol content of the extract was determined by mixing 0.5 mL aliquot (0.2 g of the extract dissolved in 20 mL 70% Acetone) with equal volume of water, 0.5 mL Folin-Cioalteu's reagent and 2.5 mL of saturates Sodium carbonate solution were subsequently added and the absorbance was measured after 40 min at 725 nm (Singleton *et al*., 1999).

Reducing Property

The reducing property of the extract was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Pulido *et al*., (2000), briefly 2.5 mL aliquot (0.5 g of the extract dissolved in 20 mL methanol) was mixed with 2.5 mL, 200 mM Sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% Potassium ferricyanide, the mixture was incubated at 50°C for 20 min, thereafter 2.5 mL, 10% Trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min, 5 mL of the supernatant was mixed with equal volume of water and 1 mL of 0.1% ferric chloride, the absorbance was later measured at 700 nm, a higher absorbance indicates a higher reducing power.

Free Radical Scavenging Ability

The free radical scavenging ability of the vegetables against DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical was also evaluated (Ursini *et al*., 1994), briefly, 1 mL aliquot (0.5 g of the extract dissolved in 20 mL methanol) was mixed with 1 mL, 0.4 mM methanolic solution containing 1, 1-diphenyl-2 picrylhydrazyl (DPPH) radicals, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

Antimicrobial Activity

The antimicrobial activity of the ethanolic extracts of the leaf was conducted using agar-diffusion methods (Ojala *et al*., 2000). The extracts were dissolved in sterile water to give a concentration range of 0.05-1.0 mg mL^{-1}. The extracts were tested using the hole-plate diffusion method. The extracts were added to the holes in the plate containing cell suspensions prepared in 0.85% saline containing about $1x10^6$ CFU mL^{-1}. The bacterial plates were incubated at 23°C for 1 h to facilitate diffusion and thenincubated at 35°C for 24 h. The antifungal test plates were refrigerated at 8°C for 1 h and then incubated at 25°C for 72 h. The effect was evaluated by measuring the diameter of the inhibitory zones.

Analysis of Data

The result of the three replicates were pooled and expressed as Mean±Standard Error (SE). Analysis of Variance (ANOVA) was used for the analysis of the data (Zar, 1984). Significance was accepted at $p \leq 0.05$.

Results and Discussion

Dietary antioxidants are beneficial because they play protective roles against oxidative stress involved in the pathogenesis of multiple disease such as cancer and cardiovascular diseases. *Ocimum gratissimum* L. (Labiatae) is widely distributed in Nigeria and it is popularly used in herbal and local soup preparation for the managament/treatment of many diseases such as upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia and also as a treatment for cough, fever and conjunctivitis. This study sought to investigate the antioxidant and antimicrobial properties of ethanolic extracts of *Ocimum gratissimum* leaf as a way of providing some information on the basis for the nutritional and medicinal properties of the vegetable.

The result of Table 1 revealed that ethanolic extract of *Ocimum gratissimum* leaf has tannin, saponin, anthraquinone and cardiac glycosides. These result compared well with the phytochemical constituent of the aqueous extracts of the leaf reported by Offiah and Chikwendu (1999), however the ethanolic extracts also have anthraquinone and cardiac glycoside which were absent in the reported aqueous extract. These different phytochemicals have been reported to have various protective and therapeutic effects essentially to prevent diseases and maintaining a state of well-being (Oboh and Ayoola, 2003). This report agrees with earlier report by Boyer and Liu (2004), in that vegetables are rich sources of phytochemicals, this phytochemicals have protective and therapeutic effect essential to preventing diseases, this they do by stimulating detoxifying enzymes in the liver to render some carcinogens and harmful chemicals harmless, scavenging free radicals produced within the body and also help the body to stimulate other chemicals that will assist the body to maintain a state of well being (Oboh, 2005).

Furthermore, the total phenol content, reducing power and free radical scavenging ability of the ethanolic extract of the leaf was determined as an index for its antioxidant property. As shown in Table 2, the total phenol content of the ethanolic extract is 3.6%, this value is higher than the methanolic extracts of mushroom (Yand *et al*., 2002), but lower than that of the ethanolic extracts of *Struchium sparjanophora* (Oboh, 2006). Phenol had been reported to be the most abundant antioxidant in plant foods, in addition, to their physiological roles in plant as an important contributor to the survival of plant species through the insurance of successful pollination and also provides plants with unpleasant taste so that possible threatening herbivores are repelled. Chu *et al*. (2002) reported that there is a correlation between the total phenol content and antioxidant activity of plant food, this they do by inhibiting oxidation of unsaturated lipids, thus preventing the formation of oxidized Low-density Lipoprotein (LDL), which is considered to induce cardiovascular disease (Amic *et al*., 2003).

Table 1: Phytochemical constituent of *Ocimum gratissimum* leaf extracts

Plant metabolite	Observation
Alkaloids	-
Saponins	+
Tannins	+
Anthraquinones	+
Cardiac glycosides	+
Phlobatannins	-

Present +, Absent -

Table 2: Antioxidant properties on *Ocimum gratissimum* leaf extracts

Antioxidant parameter	Observation
Total phenol (g/100 g)	3.6±0.2
Reducing power (OD_{700})	2.4±0.1
Free radical scavenging ability (%)	51.2±0.6

Values represent means of triplicate

Table 3: Antibacterial properties of ethanolic extracts of *Ocimum gratissimum* leaves

Concentration (mg mL^{-1})	Zone of Inhibition (mm)			
	P. aeruginosa	*Shigella dysentrae*	*Proteus* sp.	*S. aureus*
0.05	5.0±1.2^{a}	4.0±1.5^{a}	3.4±0.2^{a}	3.4±1.0^{a}
0.2	10.0±1.0^{a}	10.0±0.6^{a}	5.5±1.0^{b}	6.4±1.5^{b}
0.5	15.0±2.2^{a}	18.0±1.2^{a}	7.7±0.6^{c}	12.4±1.2^{b}
1.0	20.0±0.9^{b}	29.0±1.0^{a}	8.5±0.4^{c}	18.0±1.2^{b}

Values represent means of triplicate, Values with the same alphabet along the same row are not significantly different

Table 4: Antifungal properties of ethanolic extracts of *Ocimum gratissimum* leaves

Concentration (mg mL^{-1})	Zone of Inhibition (mm)	
	S. cerevisae	*Candida albicans*
0.05	0.0±0.0^{b}	1.5±0.2^{a}
0.2	3.0±0.2^{b}	5.0±0.5^{a}
0.5	6.0±0.5^{b}	9.0±1.2^{a}
1.0	9.0±0.4^{b}	13.0±1.0^{a}

Values represent means of triplicate, Values with the same alphabet along the same row are not significantly different

The result of the antioxidant activity of the ethanolic extracts of *Ocimum gratissimum* leaf as typified by the ability of the extract to reduce standard Ferric chloride solution and to scavenge free radicals produced by standard DPPH (1,1-diphenyl-2 picryldrazyl) free radical indicates that the reducing power (2.4 OD_{700}) and free radical scavenging ability of 51.2% were high (Table 2), however, these values were below that of ethanolic extracts of *Struchium sparjanophora* (Oboh, 2006), but higher than that of mushroom (Yang *et al*., 2002). This high reducing power and free radical scavenging ability may have possibility accounted for its use in folk medicine in the management of many diseases in the tropics. This also indicates that, just like *Ocimum basilicum L.* (Jayasinghe *et al*., 2003), that *Ocimum gratissimum* could be a good source of dietary antioxidant which could used in the management of many chronic diseases.

Furthermore, the antimicrobial properties of the extract on some *Enterobacterioceae* (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* sp., *Shigella dysentrae* and *Staphlococcuss aureus*) and fungi (*Saccharomyces cereviseae*, *Candida albicans, Penicillum* sp., *Aspergillus fumigatus, Fusarium solani* and *Aspergillus flavus*) were assessed in order to provide explanation for the used of the plant in the management of gastrointestinal disorder. The result of the study as shown in Table 3 revealed that the extracts (0.05-1.0 mg mL^{-1}) inhibited the growth of *Pseudomonads aeruginosa* (5.0-20.0 mm), *Shigella dysentrae* (4.0-29.0 mm), *Proteus* sp. (3.4-8.5 mm) and *Staphlococcuss aureus* (3.4-18.0 mm), while same dose of the extract will not inhibit the growth of *Escherichia coli.*

Likewise, same dose range of the extract inhibited the growth of *Saccharomyces cerevisae* (3.0-9.0) and *Candida albicans* (1.5-13.0 mm) as shown in Table 4. This antifungal effect agrees with earlier report by Lemos *et al.* (2005), in that hydrophilic and hydrophobic extract of the plant inhibits the growth of *Cryptococcus neoformans.* However, the extract did not inhibit the growth of *Aspergillus fumigatus, Fusarium solanim* and *Aspergillus flavus.* From this result, it is obvious that the extract appears to be more active against the bacteria than fungi; likewise the inhibitory effects of the extracts were dose dependent on both the bacteria and fungi. It is also obvious from the study that the extract has its highest inhibitory effect on *Shigella dysentrae,* this result agree will earlier report by Iwalokun *et al.* (2004), in that aqueous extract of *Ocimum gratissimum* inhibits the growth of *Shigella* sp., however the ethanolic extracts of the leaf had a higher inhibitory effect on *Shigella dysentrae* than the aqueous extract, but its inhibitory effect on *Shigella dysentrae* and other bacteria is lower than that of essential oil from the plant (Iwalokun *et al*., 2003). This finding will go a long way in explaining the use of the leaf of *Ocimum gratissimum* for the preparation of soup/or infusion in the

management and prevention/treatment of dysenteries, whose causative agent is *Shigella dysentrae*. The modes of action of the extract are dose-dependant and bacteriostatic and fungastatic. These findings agree with earlier finding of Oboh and Ayoola (2003) on the mode of action of ethanolic extracts of *Alchornea laxiflora* leaves on some *Enterobacterioceae* as well as findings of Oboh (2001) on the mode of action of onion and garlic volatile oil on some *Enteriobacterioceae*.

The basis for the bacteriostatic and fungistatic activities of the extract cannot be categorically stated; however this could be attributed to the presence of some secondary metabolites like saponin, tannin, anthraquinone and cardiac glycosides which had been reported to have antimicrobial and therapeutic activity (Oboh and Ayoola, 2003; Oboh *et al*., 1998). It could therefore be concluded that part of the reasons for the use of *Ocimum gratissimum* leaves in folk medicine against gastrointestinal disorders and other chronic diseases could be as a result of its antioxidant and antimicrobial property which is hinged on the array of pharmacologically active phytochemicals present in the vegetable leaves.

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