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Full Length Research Paper

Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*

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Extracts from the leaves of *Ocimum gratissimum* were investigated for phytochemical constituent and antioxidant activity. Tests for tannins, steroids, terpenoids, flavonoids and cardiac glycosides were positive in both methanolic and aqueous extracts. The methanolic extract of *O. gratissimum* had a DPPH scavenging activity of 84.6% at 250 µg/ml and a reductive potential of 0.77 at 100 µg/ml. These values were comparable with those of gallic acid, 91.4% at 250 µg/ml and ascorbic acid, 0.79 at 60 µg/ml as standards for DPPH scavenging activity and reductive potential, respectively. These findings suggest that the rich phytochemical content of *O. gratissimum* and its good antioxidant activity may be responsible for its popular and wide traditional use.

Key words: *ocimum gratissimum*, phytochemicals, antioxidant activity and reductive potential.

INTRODUCTION

Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. These complex materials are refer-red to as secondary metabolites. Plants secondary meta-bolites have recently been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease-inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992; Farombi et al., 1998). Antioxidants protect other mole-cules (*in vivo*) from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the aetiology of many diseases and in food deterioration and spoilage (Halliwell and Gutteridge, 1992; Kasaikina, 1997; Farombi, 2000; Koleva et al., 2000).

Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce de-definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). *Ocimum gratissimum* Linn (Labiatae) is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linalool have been extracted from the oil (Sulistiarini, 1999). Essential oils from the plant have been reported to possess an interesting spectrum of anti-fungal properties (Dubey et al., 2000). The antinocicep-tive property of the essential oil of the plant has been reported (Rabelo et al., 2003). The whole plant and the essential oil are used in traditional medicine especially in Africa and India. The essential oil is also an important insect repellant. *O. gratissimum* is germicidal (Nakamura et al., 1999; Pessoa et al., 2003; Holets et al., 2003) and has found wide use in toothpastes and mouth washes as well as some topical ointments. It is used as an excellent gargle for sore throats and tonsillitis. It is also used as an expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animals and man (Fakae, 2000; Chitwood, 2003). In addition, *O. gra-tissimum* carminative properties make it a good choice for upset stomach. It is used as an emetic and for hemor-rhoids. The plant is also used for the treatment of rheu-matism, paralysis, epilepsy, high fever, diarrhea, suns-troke, influenza, gonorrhea and mental illness (Dhawan et al., 1977; Oliver, 1980; Abdulrahman, 1992; Osifo,

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1992; Sofowora, 1993; Sulistiarini, 1999) . In addition, the plant is used as a spice and condiment in the southern part of Nigeria.

The present work has been designed to evaluate the antioxidant potential of extracts from the leaves of *O. gratissimum* and to explore the basis for its traditional use.

MATERIALS AND METHODS

Chemicals

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical, gallic acid, ascorbic acid and Folin-Ciocalteau reagent were obtained from Sigma-Aldrich, USA. All other chemicals and reagents used were of analytical grade.

Plant materials

Leaves of *O. gratissimum* were collected from a farmland in Akure, South-Western Nigeria and identified at the department of Crop, Soil and Pest Management, Federal University of Technology, Akure. They were air dried, packed in paper bags and stored. The dried leaves were pulverized and 200 g of the pulverized sample was extracted with 500 ml of 80% methanol by maceration for 72 h. The methanolic extract was concentrated in a rotary evaporator, lyophilized and thereafter preserved for further use. An aqueous extract was also prepared from the pulverized sample for the purpose of comparison of the phytochemical constituents with that of the methanolic extract.

Phytochemical screening

Chemical tests were carried out on the aqueous and methanolic extracts for the qualitative determination of phytochemical constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

Total phenolic content

Total phenolic content was determined using Folin-Ciocalteau reagent as previously described (McDonald et al., 2001). Total phenol value was obtained from the regression equation: y = 0.0055x + 0.1139 and expressed as mg/g gallic acid equivalent using the formula, C = cV/M; where C = total content of phenolic compounds in mg/g GAE, c = the concentration of gallic acid (mg/ml) establishhed from the calibration curve, V = volume of extract and m = the weight of pure plant methanolic extract (g).

DPPH radical scavenging activity

The ability of the extract to scavenge DPPH radical was determined according to the method described by Mensor et al. (2001). Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5 µg/ml in methanol. 1 ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%) using the formula:

AA% = 100 – [(Abs sample – Abs blank) × 100]/Abs control

Methanol (1.0 ml) plus extract solution (2.5 ml) was used as a blank. 1 ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control. Solution of gallic acid served as positive control.

Reductive potential

This was determined according to the method of Oyaizu (1986). Different concentrations of the methanolic extract of *O. gratissimum* (20, 40, 60 and 100 µg/ml) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50˚C for 20 min. Trichloroacetic acid (10%, 2.5 ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 ml) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicates higher reductive potential.

Statistical analysis

Data were expressed as mean ± SEM. A one-way analysis of variance was used to analyze data. $P<0.5$ represented significant difference between means (Duncan's multiple range test).

RESULTS AND DISCUSSION

Table 1 shows the phytochemicals detected in *O. gratissimum* leaf extract. Tests for tannins, steroids, terpenoids, flavonoids and cardiac glycosides were positive in both methanolic and aqueous extracts. Anthraquinones were detected only in the aqueous extract while alkaloids were detected only in the methanolic extract. Saponins were not detected in both extracts. These phytochemicals may be responsible for the medicinal value of *O. gratissimum*. The total phenolic content in the methanolic extract was 5.68 ± 0.06 mg/g GAE. Phenolics are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products. The result of the DPPH scavenging activity of *O. gratissimum* extract compared to that of gallic acid (GA) is shown in Figure 1. Both showed a dose-dependent antioxidant activity. The AA% of GA was remarkably higher than that of *O. gratissimum* at lower concentrations but significant differences between them seem to be less conspicuous at higher concentrations. The reductive potentials of *O. gratissimum* extract and ascorbic acid (AA) were also dose-dependent (Figure 2. The reductive potential of AA was clearly higher than that of *O. gratissimum* at all concentrations except the least (20 µg/ml). However, it should be noted that the reductive potential of *O. gratissimum* was still appreciable.

Results from the present investigation shows that *O. gratissimum* is rich in phytochemicals. Specific biologically important compounds have been identified in extracts from the plant by previous workers (Sulistiarini, 1999; Dubey et al., 2000; Holets et al., 2003). The present work also reveals that the extract from the leaves of *O. gratissimum* possesses good antioxidant potential presumably because of its phytochemical constituents (Thabrew et al., 1998; Halliwell and Gutteridge, 1992). The DPPH scavenging activities of OG showed a good correlation with its reductive potentials. These facts justify the medicinal use of the plant for the treatment of various maladies (Dhawan et al., 1977; Oliver, 1980). However,

Table 1. Phytochemicals in methanolic and aqueous leaf extract of *O. gratissimum*.

Phytochemicals	Methanolic extracts	Aqueous extracts
Alkaloids	+	-
Saponins	-	+
Tannins	+	+
Phlobatannins	+	+
Anthraquinones	-	+
Steroids	+	+
Terpenoids	+	+
Flavonoids	+	+
Cardiac glycosides		
With steroidal ring	+	+
With deoxy – sugar	-	+

+ = Present
- = Absent

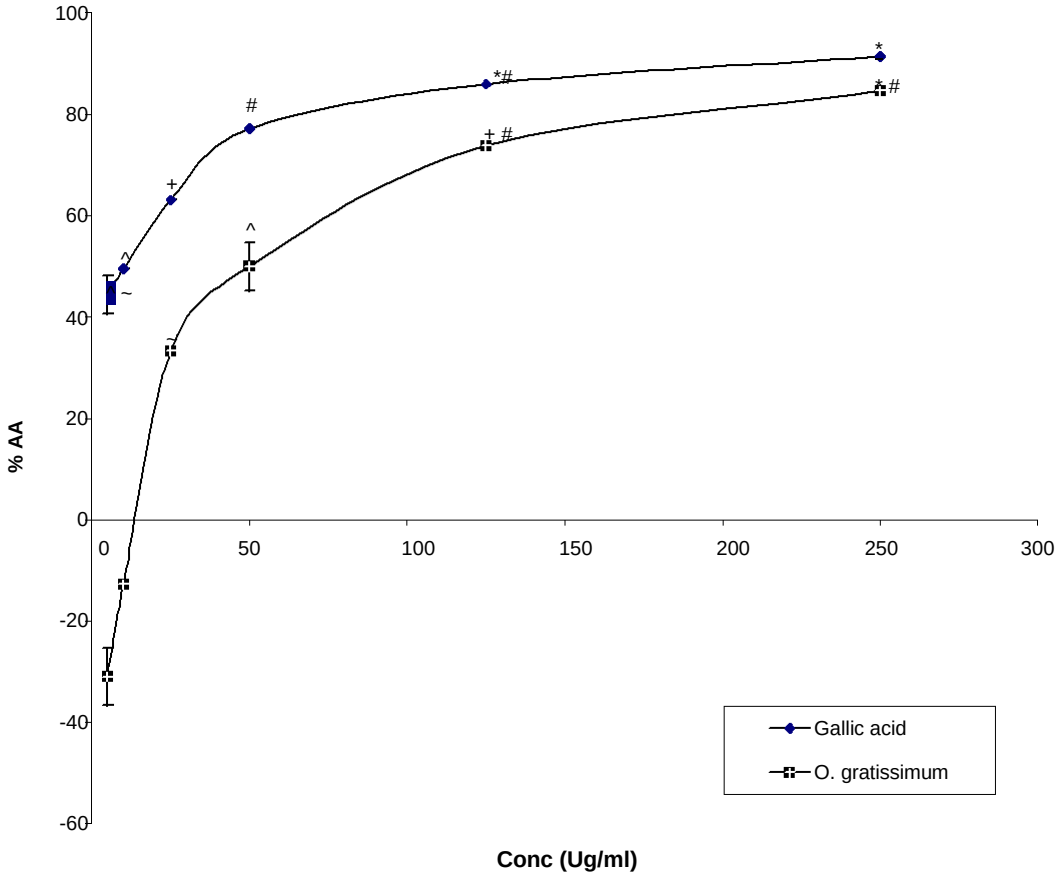

Figure 1. Dose-dependent DPPH scavenging activity of *O. gratissimum* leaf extract and gallic acid. Values sharing a common symbol are not significantly different (P>0.05). Significantly different at P<0.05: GA_{250} vs GA_{50}; GA_{50} vs GA_{25}; GA_{25} vs GA_{10}; GA_{25} vs OG_{50}. Significantly different at P<0.01: GA_{250} vs OG_{125}; GA_{10} vs OG_{25}; OG_{50} vs OG_{25}. Other values significantly different at P<0.001.

further work is necessary to ascertain the clinical safety of extracts from the plant (Effraim et al., 2001) and to determine appropriate concentration for therapy so as to safeguard the health of the teeming mass of traditional

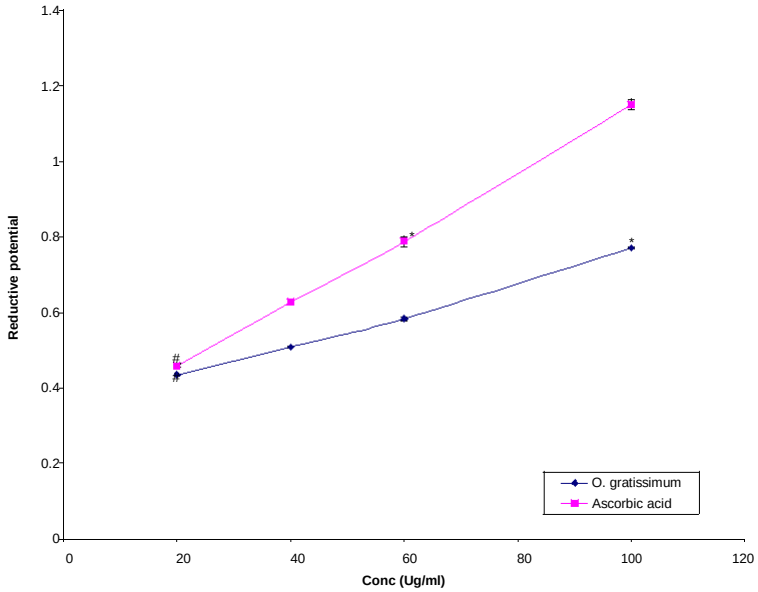

Figure 2. Dose-dependent reductive potential of *O. gratissimum* leaf extract and ascorbic acid. Values sharing a common symbol are not significantly different. OG_{60} significantly differ-ent from AA_{40} ($P<0.05$); OG_{40} significantly different from AA_{20} ($P<0.01$). All other values are significantly different ($P<0.001$).

users who more often that not, do not take these factors into consideration.

REFERENCES

Abdulrahman F (1992). Studies in natural products: The *Moraceae* in African traditional medicine and management of psychiatry in Bornu State. M. Sc thesis, Department of Chemistry, University of Maiduguri Chitwood DJ {2003). Phytochemical based strategies for nematode control. Annual Review of Phytopathology 40: 221-249.

Dhawan BN, Patnik GR, Rastogy RAT, Singh KK, Tandol TS (1977). Screening of Indian plants for biological activity. YL India Exp. B. 15: 108.

Dubey NK Tiwari TN Mandin D Andriamboavonjy H Chaumont JP (2000). Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). Fitoterapia 7(15): 567-569.

Effraim KD Jacks TW Sodipo OA (2001). Histopathological studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of rabbit. Afr. J. Biomed. 6: 21-25.

Fakae BB Campbell AM Barrett J, Scott IM, Teesdale-Spittle PH,Liebau E, Brophy PM (2000). Inhibition of glutathione-S -transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytother. Res. 1148: 630-634.

Farombi EO, Nwamkwo JO, Emerole GO (1998). Effect of methanolic extract of browned yam flour diet on 7,12-Dimetylbenzanthracene (DMBA) and 3-methylcholanthrene (3-MC) induced toxicity in the rat. Proc. Fed. Afr. Soc. Biochem. Mol. Biol. 1: 5-10.

Farombi EO (2000). Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. Pharmacol. Res 42: 75-80.

Halliwell B, Gutteridge JMC (1992). Free radicals, antioxidants and human diseases: where are we now? J. Lab. Clin. Med. 119:598-620.

Harborne JB (1973). Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapman and Hall, London.

Hill AF (1952). Economic Botany. A textbook of useful plants and plant products. 2 nd edn. McGraw-Hill Book Company Inc, New York.

Holets FB, Ueda-Nakamura T, Filho BPD, Cortez DAG, Morgado-Diaz JA, Nakamura CV (2003). Effect of essential oil of *Ocimum gratissimum* on the trypanosomatid *Herpetomonas samuelpessoai.* Act. Protonzool 42: 269-276.

Kasaikina OT, Kortenska VD, Marinova EM, Rusina IF Yarisbheva NV (1997). Russ. Chem. Bull. 46: 1070-1073.

Koleva II, Niederlander HAG, Van Beek TA (2000). An online HPLC method for detection of radical scavenging compounds in complex mixtures. Anal. Chem. 72: 2323-2328.

McDonald S, Prenzler PD, Autolovich M Robards K (2001). Phenolic content and antioxidant activity of olive extracts. Food Chemistry, 73: 73-84.

Mensor LI, Menezes FS, Leitao GG, Reis AS, dos Santos T, Coube C S, Leitao SG (2001). Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy research, 15: 127-130.

Nakamura CV, Nakamura TU, Bando E, Melo AJN, Cortez DAG, Dias Filho BP (1999). Antibacterial activity of *Ocimum gratissimum* essential oil. Mem. Inst. Oswaldo Cruz 94: 675-678.

Oyaizu M, (1986). Studies on product of browning reaction prepared from glucose amine. Japan Journal of Nutrition, 44: 307-315.

Pessoa LM, Morais SM, Bevilaqua CML, Luciano JHS (2002). Antihelmintic activity of essential oil of *Ocimum gratissimum* Linn and eugenol against *Haemoachus contortus*. Vet. Parasitol. 109: 59-63.

Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA Criddle DN, (2003). Antinociceptive properties of the essential oil of *Ocimum gratissimumI* L. (Labiatae) in ice. Bra. J. Med. Biol. Res. 36: 521-524.

Sofowora LA (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibaban. pp. 55-71.

Sulistiarini D, Oyen LPA, Nguyen Xuan Dung (1999). *Ocimum gratis-simum* L. In: Plant Resources of South-East Asia. No. 19: Essential-oils Plants. Prosea Foundation, Bogor, Indonesia. pp. 140-142.

Thabrew M I, Hughes RD, McFarlane IG (1998). Antioxidant activity of *Osbeckia aspera*. Phytother. Res. 12: 288-290.

Trease GE, Evans WC, (1989). Trease and Evans' Pharmacognosy: A Physician's Guide to Herbal Medicine. 13th Edition, Bailliere Tindall London.