ISSN - 2277 - 0593

Journal of Natural Sciences, Engineering and Technology

EVALUATION OF THE PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL PROPERTIES OF CRUDE METHANOLIC EXTRACT OF LEAVES OF *Ocimum gratissimum L.*

G.I. AMEH

Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria. P.O. Box 1062, Nsukka Enugu State.
E-mail: g_ameh@yahoo.com **Tel:** +2348035090331

ABSTRACT

Methonolic extract of the leaves of *Ocimum gratissimum* L. was investigated for its phytochemical composition and antimicrobial properties. Phytochemical screening of the leaf extract revealed the presence of carbohydrates, proteins and terpenoids in high concentrations (+++). Reducing sugars, glycosides and tannins occurred in moderate amounts (++) while saponins, flavonoids and steroids were present in low concentrations (+). The leaf extract was devoid of alkaloids, resins, oils and acidic compounds (-). *Bacillus subtilis* was the only microorganism sensitive to the leaf extract and had an inhibition zone diameter of 12.5mm. The minimum inhibitory concentration (MIC) of the extract against *B. subtilis* was 100 mg/ml. The antibacterial potential of this plant part which can be exploited in the future for its bioactive constituents has thus been demonstrated.

Keywords: *Ocimum gratissimum, antimicrobial activity, Phytochemical, Minimum inhibitory concentration.*

INTRODUCTION

The use of medicinal plants in most developing countries of the world for maintenance of good health care has been widely recognized (UNESCO, 1996). Traditional healers that claim the efficacies of medicinal plants for cure of several diseases are on the increase. Herbal medicine has since been used either as dietary supplement or for therapeutic purposes. Medicinal plants contain bioactive constituents that have been exploited in traditional medicine for the treatment of various ailments (Adebajo *et al.,* 1983). Several authors have reported that plants contain a wide variety of bioactive constituents (Cowman, 1999, Edeoga *et al.,* 2005). These bioactive constituents of plants have been demonstrated to possess antimicrobial properties. Banso and Adeyemo (2007) reported the antimicrobial activity of alkaloids, glycosides, flavonoids and tannins from *Dracaena mannii* stem bark. Report by Ogbonna *et al.,* (2007) indicates that the seed extract of *Ricinus communis* had antifungal activity on *Trichophyton sp.* and *Candida albicans* but had no antibacterial effects on *Staphylococcus aureus* and *Escherichia coli*. The leaf extract of *Ocimum gratissimum* was shown to have antimicrobial activity (Junaid, 2006, Mbata and Saikia, 2008).

Ocimum gratissimum is an aromatic medicinal

plant that belongs to the *Lamiaceae* family. It is a shrub found commonly around village huts and in gardens (Iwu, 1993). The leaves are used as vegetable and can be rubbed between palms and sniffed as treatment for blocked nostrils (Kokwara, 1993). *O. gratissimum* is used in the treatment of different diseases such as cough, diarrhoea, headache, fever, skin infections, pneumonia, barrenness, convulsion and regulation of menstruation (Harjula, 1980; Onajobi, 1986). Following the numerous uses of *O. gratissimum* in herbal medicine, a knowledge of the biological activity will provide valuable scientific data concerning the plant. Few studies have been carried out on *O. gratissimum* e.g. Mbata and Saikia, 2008, but the study was based on the ethanolic leaf extract and the test organism was *Listeria monocytogenes*. Therefore, the aim of this study was to investigate the phytochemical composition and antimicrobial properties of crude methanolic extract of the leaves of *O. gratissimum.*

MATERIALS AND METHODS

Collection of samples

Fresh leaves of *Ocimum gratissimum* were collected from the plants growing around a village house in Nsukka, Enugu State and authenticated by Mr. A.O. Ozioko, a Taxonomist of Bioresource Development and Conservation Programme (BDCP), Nsukka.

Preparation of plant material

The leaves were air-dried and grated in a mechanical greater. The grated material was sieved to obtain a fine powder. This was stored in a plastic container for further use.

Phytochemical tests

The phytochemical screening of the powdered leaf sample of *O. gratissimum* was carried out using the procedures and methods of Trease and Evans (1989).

Preparation of plant extract

Methanol extraction of the leaves of *O. gratissimum* was carried out using the procedure described by Harbone (1994). 25 g of the powered sample was Soxhlet extracted using 250 ml of 95% methanol. The exercise lasted for 24 hrs. The dried extract was then stored in a refrigerator at 4^0C until required for use. Concentrations of 400, 200, 100, 50, 25, 12.5, 6.25 mg/ml of the extract were prepared by dilution.

Test organisms

Clinical isolates of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger* and *Candida albicans* were obtained from Pharmaceutical Microbiology Laboratory of University of Nigeria, Nsukka and used in this study.

Sensitivity test

The antimicrobial tests of the plant extract were carried out on the test isolates using agar-diffusion method described by Boakye-Yiadom (1979). Nutrient agar (NA) and Sabouraund dextrose agar (SDA) were prepared for the tests. *E. coli, B. subtilis, S. aureus and P. aeruginosa* were inoculated on NA while *A. niger* and *C. albicans* were inoculated on SDA and spread uniformly using a sterile glass spreader. Cavities of 1 cm diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were removed with sterilized forceps. In each of the cavities was introduced 0.1 ml of the plant extract. One of the cavities was filled with 0.1 ml of methanol to serve as control. The agar plates were allowed to stand for 1 hr on the bench for diffusion to occur before the growth of the organisms commenced, and incubated at 37^0C for 24 hrs. The average of the readings from the cavities

except control was taken as the zone of inhibition of the microorganisms at that particular concentration.

Minimum inhibitory concentration (MIC)
The minimum inhibitory concentration of the extract was determined according to the technique of Baron and Finegold (1990). Standardized suspension of the test organism was inoculated into a series of sterile tubes of nutrient broth containing different concentrations of the leaf extract and incubated at 37^{0}C for 24 hrs. The MIC was recorded as the least concentration that inhibited the growth of the test organism.

RESULTS

The leaf extract of *Ocimum gratissimum* showed positive results for carbohydrates, reducing sugars, glycosides, saponins, tannins, flavonoids, proteins, steroids and terpenoids (Table 1). However, the extract of the plant material was devoid of alkaloids, resins, oils and acidic compounds. Carbohydrates, proteins and terpenoids occurred in high concentrations. Reducing sugars, glycosides and tannins were present in medium concentrations while saponins, flavonoids and steroids were found in low concentrations (Table 1).

Table 2 shows the results of the sensitivity tests of the microorganisms to the leaf extract. Out of the six microorganisms assayed, only *Bacillus subtilis* was sensitive to the methanolic leaf extract of *O. gratissimum*. It had an inhibition zone diameter of 12.5 mm (Table 2).

The minimum inhibitory concentration (MIC) of the leaf extract of *O. gratissimum* against *Bacillus subtilis* is shown in Table 3. The MIC result indicated that methanolic extract of the leaf had MIC of 100mg/ml.

DISCUSSION

The phytochemical composition and antimicrobial properties of the leaves of *Ocimum gratissimum* have been studied. The positive results for carbohydrates, reducing sugars, glycosides, saponins, tannins, flavonoids, proteins, steroids and terpenoids confirms the presence of these secondary metabolites in the leaf extract of *O. gratissimum*. Similar secondary metabolites have been reported on *Raphia hookeri* (Akap and Usoh, 2004), *Euphorbia heterophylla* (Falodun *et al.,* 2006), *Dracaena mannii* (Banso and Adeyemo, 2007) and *Garcinia kola* (Adegboye *et al.,* 2008). Antimicrobial effects of plant extracts have been attributed to the presence of these secondary metabolites (Nweze *et al.,* 2004; Oboh and Masodje, 2009). The presence of these metabolites in the investigated plant part account for its usefulness as a medicinal plant.

The zone of inhibition observed in this study is comparable to those obtained in other studies (Junaid, 2006; Adegboye *et al.,* 2008). Various plants that are rich in alkaloids, tannins, glycosides, flavonoids, saponins have been shown to possess antimicrobial activity against a number of microorganisms. Adebajo *et al.* (1983) reported the presence of tannins, glycosides and alkaloids from leaf extract of *Eugenia uniflora* and that the methanolic extract of the plant was active against *E. coli, P. vulgaris, K. pneumoniae* and *A. niger.* Adiguzel *et al.* (2005) showed that methanol and ethanol leaf extracts of *Ocimum basilicum* inhibited *C. albicans* and *B. subtilis*. In this study, the leaf extract showed inhibitory activity against only one bacteria (*B. subtilis*) but did not show any activity against fungi. This could probably be due to the biochemical differences in the structure within the cell components of bacteria and fungi.

Table 1: Phytochemical characteristics of the leaf extract of *Ocimum gratissimum*

Active Ingredients	Extract
Carbohydrates	+++
Reducing sugars	++
Alkaloids	-
Glycosides	++
Saponins	+
Tannins	++
Flavonoids	+
Resins	-
Proteins	+++
Oils	-
Steroids	+
Terpenoids	+++
Acidic compounds	-

-absent, + low concentration, ++ medium concentration, +++ high concentration.

Table 2: Sensitivity test for microorganisms to the methanolic extract of *Ocimum gratissimum*

Test Organism	Inhibition Zone Diameter (mm)
* *Escherichia coli*	0.0
* *Bacillus subtilis*	12.5
* *Staphylococcus aureus*	0.0
* *Pseudomonas aeruginosa*	0.0
= *Aspergillus niger*	0.0
= *Candida albicans*	0.0

* Bacteria = Fungi

Table 3: Minimum inhibitory concentration (MIC) of the leaf extract against *Bacillus subtilis*

Test Organism	Zone of Inhibition (mm)						
	Concentration (mg/ml) of the extract						
	400	200	100	50	25	1.25	6.25
B. subtilis	12.5	6.0	2.5	NI	NI	NI	NI

NI = No inhibition

Fungi contain sterols in their cell membrane while this is absent in bacteria. According to Lorian (2000), antifungal agents have different mode of action from antibacterial agents.

The MIC observed in this study is similar to that reported by Nwadiaro and Nwachukwu (2007) on *Ceiba pentandra*. However, the MIC recorded by Mbata and Saikia (2008) on the ethanolic leaf extract of *O. gratissimum* against *Listeria monocytogenes* was lower than what was observed in this study. The results of this study demonstrate that the leaf extract of *O. gratissimum* contain a number of phytochemicals and has antibacterial activity. The isolation and characterization of the specific secondary metabolites contained in this plant part will be of interest.

REFERENCES

Adebajo, A.O., Adewumi, C.O., Essien, E.E. 1983. Anti-infective agent of higher plants. International Symposium of Medium Plants. 5th ed. University of Ife, Nigeria. pp 152 – 158.

Adegboye, M.F., Akinpely, D.A., Okoh, A.I. 2008. The bioactive and phytochemical properties of *Garcinia kola* seed extract on some pathogens. *Afri. J. Biotech.,* 7(21): 3934 – 3938.

Adiguzel, A., Sahin, F., Karaman, I., Gulluce, M. 2005. Antimicrobial effects of *Ocimum basilicum* extract. *Turk. J. Biology,* 29: 155-160.

Akpan, E.J., Usoh, I.E. 2004. Phytochemical screening and effect of aqueous root extract of *Raphia hookeri* on metabolic clearance rate of ethanol in rabbits. *Biokemistri,* 16(1): 37 – 42.

Baron, J.E., Finegold, S.M. 1990. Method for testing antimicrobial effectiveness. In: Bailey Scotts Diagnostic Microbiology. Mosby, C.V. (ed.), Missouri. pp 171 – 194.

Banso, A., Adeyemo, S.O. 2007. The phytochemical and antimicrobial evaluation of ethanolic extract of *Dracaena mannii*. *Nig. J. Biotech*., 18(1–2): 27 – 32.

Boakye – Yiadom, I.K. 1979. Antimicrobial activity of two flavonones isolated from the Cameroonian plant *Erythrinna sigmoidea. Plant Medica,* 54(2): 126 – 212.

Cowman, M.N. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Review,* 12(4): 564 – 582.

Edeoga, H.O., Okwu, D.E. Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian Medicinal Plants. *Afri. J. Biotech.,* 4(7): 685 – 688.

Falodun, A., Okunrobo, L.O., Uzoamaka, N. 2006. Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla. Afri. J. Biotech.,* 5(6): 529 – 531.

Harbone, N.V. 1994. Phytochemical methods. A guide to modern techniques of plant analysis. 2nd ed. Chapman and Hall, London. p. 425.

Harjula, F.A. 1980. Characteristics of chemotypes as found in the chemical composition of *Ocimum gratissimum*. *Pharmacology,* 81(2): 61 – 80.

Iwu, E.C. 1993. Ethnobotanical importance of some Nigerian plants. *Jour. Ethnomedicine,* 55: 893 – 906.

Junaid, S.A. 2006. The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *African J. Biotech.,* 5(22): 2315 – 2321.

Kokwara, V.D. 1993. Importance of *Ocimum gratissimum* on blocked nostrils. *Nig. J. Pharmacology,* 100: 560 – 570.

Lorian, V. 2000. Antibiotic in Laboratory Medicine. Wolters Kluwer Health, Philadelphia. 832 p.

Mbata, T.I., Saika, A. 2008. Antibacterial activity and phytochemical screening of crude ethanolic extract of leaves of *Ocimum gratissimum* on *Listeria monocytogenes*. *The Internet J. Microbiology,* 4(2): 1 – 13.

Nwadiaro, P.O., Nwachukwu, I. 2007. Inhibition of pathogenic fungi by ethnobotanical extracts of *Cymbopogon citrates, Ceiba pentandra* and *Loranthus bengwelensis. Nig. J. Biotech.,* 18(1-2): 61 – 65.

Nweze, E.I., Okafor, J.I., Njoku, O. 2004. Antimicrobial activities of methanolic extract of *Trema guineensis and Morinda incida* used in Nigeria Herbal medicinal practice. *J. Biol. Res. Biotech.,* 2(1): 39 – 46.

Oboh, F.O.J., Masodje, H.I. 2009. Nutritional and antimicrobial properties of *Ocimum gratissimum leaves. J. Biol. Sciences.,* 9(4): 377 – 380.

Ogbonna, A.I., Makut, M.D., Gyar, S.D., Adamu, E.U. 2007. Antimicrobial activity of the ethanolic extract of the seeds of *Ricinus communis. Nig. J. Biotech.,* 18(1-2): 40 – 43.

Onajobi, F.D. 1986. Smooth muscle contracting lipid soluble principles in chromatographic fractions of *Ocimum gratissimum. J. Ethno-pharmacol,* 18: 3 – 11.

Trease, G.E., Evans, W.C. 1989. Pharmacognosy. 13th ed. Bailliere Tindall Ltd., London. pp 350 – 389.

UNESCO 1996. Culture and Health Orientation Texts. World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – 1996, Paris, France p. 129.

(Manuscript received: 29th October, 2009; accepted:, 29th August, 2011).