Journal of Agricultural Technology 2010 Vol.6(1): 57-65
Available online http://www.ijat-rmutto.com
ISSN 1686-9141

The extract of *Ocimum gratissimum* on the dehydrogenase activities to clinical isolates of *Escherichia coli* and *Staphylococcus aureus*

Akujobi, C.O.[1*], Ogbulie, J.N.[1] and Njoku, H.O.[2]

[1]Microbiology Department Federal University of Technology, PMB 1526, Owerri, Imo State, Nigeria.
[2]Microbiology Department, University of Port Harcourt, PMB 5323, Port Harcourt, Rivers State, Nigeria.

Akujobi, C.O., Ogbulie, J.N. and Njoku, H.O. (2010). The extract of *Ocimum gratissimum* on the dehydrogenase activities to clinical isolates of *Escherichia coli* and *Staphylococcus aureus*. Journal of Agricultural Technology 6(1): 57-65.

Inhibitions of dehydrogenase activities of clinical bacterial isolates by leaf extract of *Ocimum gratissimun* were investigated. The isolates were *Escherichia coli* and *Staphylococcus aureus*. The cultures were exposed to extract concentrations of 0-2,500 µg/ml in a nutrient broth-glucose-TTC medium. The responses of the bacterial isolates varied with extract concentrations. In both isolates, dehydrogenase activities were progressively inhibited with increasing concentration of extract. The IC_{50} were 280.19 ± 18.2 and 397.08 ± 23.1 while IC_{100} were 2,201.75 ± 21.1 and 2,474.60 ± 40.3 for *E.coli* and *S.aureus*, respectively. The findings may be of clinical relevance and further confirms the antibacterial properties of *Ocimum gratissimum.*

Introduction

Ocimum gratissimum is a valuable multi purpose medicinal plant, which belongs to the family Lamiaceae, and is distributed in tropical and warm regions. It is commonly used in the treatment of various diseases such as upper respiratory tract infections, diarrhea, headache, fever, ophthalmic and skin diseases and pneumonia. (Gopi *et al.*, 2006). Extracts of the plant contains antimicrobial (Adebolu and Oladimagi, 2005), antibacterial (Nakamura *et al.*, 1999), antifungal (Lemos *et al.*, 2005), antimalarial (Ezekwesili *et al.*, 2004) and antiprotozoal (Holetz1 *et al.*, 2003) activities. The active compounds present as volatile oil from the leaves consist mainly of thymol (32-65%) and eugenol (Pino *et al.*, 1996). It also contains xanthones, terpenes and lactones (Ijaduola *et al.*, 1980), together with cardiac glycosides, saponnins, tannins and alkaloids (Akujobi *et al.*, 2004).

[*]Corresponding author: Akujobi, C.O.; e-mail address: campbell205@yahoo.com

Measurement of microbial enzyme activity has been used in the assessment of ecotoxicological impacts of environmental substrates. In this regard, dehydrogenase activity has been widely used. The dehydrogenase assay is an effective primary test for assessing the potential toxicity of metals to soil microbial activities (Aoyama and Nagumo, 1997; Chander and Brookes, 1995; Kelly and Tate, 1998; Rogers and Li, 1985), toxicity of metals to planktonic (Nweke *et al.,* 2006) and heterotrophic (Nweke *et al.,* 2007) bacteria from tropical river sediments. Toxicity of plant extracts to pathogenic bacteria has been assessed using the dehydrogenase assay (Nwaogu *et al.,* 2007; Nwaogu *et al.,* 2008; Alisi *et al.,* 2008).

This work was undertaken to investigate the effect of extracts of *Ocimum gratissimum* on the dehydrogenase activities of clinical isolates of *E. coli* and *S. aureus* to validate or otherwise the claim of herbalists who use the leaf extracts as antimicrobial herbal remedy. The study will make for more economic and optimal use of the plant in alternative medicine.

Materials and methods

Sample collection and preparation

The leaves of *Ocimum gratissimum* were collected from Nguru Ngor Okpala, Imo State, Nigeria. The plant was identified by Dr F.N. Mbagwu, a plant taxonomist of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The fresh leaves were sun dried for four days. The dried leaves were pulverized using mechanical grinder. About 100 g of the leaf powder was boiled in 200 ml of water and filtered using Whatmann number 1 filter paper. The filtrate was evaporated to dryness in a rotary evaporator (Model type 349/2, Corning ltd). The extracts were stored at 4 ^{0}C.

Two clinical bacterial strains, *E .coli* and *S. aureus*, were obtained from the Pathology Department of Federal Medical Center, Owerri, Nigeria. They were isolated and purified on Mueller-Hinton agar plates, and identified using their cultural, morphological and biochemical characteristics (Holt *et al.,* 1994).

The bacterial isolates were grown to mid exponential phase in nutrient broth (Lab M) on a rotary incubator (150 rpm) at room temperature (28 ± 2 ^{0}C). The cells were harvested by centrifugation at 6000 rpm for 8 min and washed thrice with distilled water. The washed cells were re-suspended in distilled water and the turbidity adjusted to an optical density of 0.85 at 500 nm. An aliquot of 0.3 ml of the cell suspension was used as inoculum in the dehydrogenase activity assay. The dry weight of the cells was determined by drying a 10 ml aliquot of cell suspension in a pre-weighed crucible to constant weight in an oven at 110 ^{0}C.

Antimicrobial activity evaluation

The dehydrogenase assay method as described by Alisi *et al.* (2008) and Nweke *et al.* (2007) was adopted for the study. The dehydrogenase activity (DHA) was determined using 2, 3, 5-triphenyltetrazolium chloride (TTC) as the artificial electron acceptor, which was reduced to the red coloured triphenylformazan (TPF). The assay was done in 4 ml volumes of nutrient broth-glucose-TTC medium supplemented with varying concentrations (0-2500 µg/ml) of the leaf extract in separate screw-capped test tubes. About 0.3 ml volume of the bacterial suspension was inoculated into triplicate glass tubes containing 2.5 ml of phosphate-buffered (pH 6.8) nutrient broth-glucose medium supplemented with varying concentrations of the extract solution. They were incubated in a rotary incubator (150 rpm) at room temperature (28 ± 2^0C) for 30 min. Thereafter, 1 ml of 0.4% (w/v) TTC in deionized water was added to each tube to obtain final extract concentrations of 0, 20, 50, 100, 200, 400, 800, 1400, 2000 and 2500 µg/ml in different test tubes. The control consisted of the isolates and the media without *O. gratissimum* extracts.

The reaction mixtures were further incubated statically at room temperature (28 ± 2^0C) for 16 h. The triphenylformazan produced was extracted in 4 ml of amyl alcohol and determined spectrophotometrically at 500 nm. The amount of formazan produced was determined from a standard dose-response curve [0-20 µg/ml TPF (Sigma) in amyl alcohol]. Dehydrogenase activity was expressed as mg of TPF formed per mg dry weight of cell biomass per hour. Inhibition of dehydrogenase activity in the isolates by *O. gratissimum* extract was calculated relative to the control. The percentage inhibition for each of the test organisms were linearized against the concentrations of the extracts using gamma parameter (r^-) [r^- = % inhibition/ (100- % inhibition)] (Kim *et al.*, 1994). The toxicity threshold concentrations (IC_{50}) were determined from the linear regression plots. The total inhibitory concentrations (IC_{100}) were extrapolated from the plot of the inhibition data.

Statistical analysis

Data obtained from this study were analyzed using a two-way analysis of variance (ANOVA) and values for $P<0.05$ were considered statistically significant.

Results and discussion

Two bacteria species comprising of one gram-negative (*E. coli*) and one gram-positive (*Staphylococcus aureus*) were isolated. Results obtained from

the control samples showed that these organisms were able to reduce TTC to the red formazan at variable rates and extent (Table 1). The gram-negative *E. coli* had higher rates of dehydrogenase activity than the gram-positive *S. aureus*. This variation may be due to differences in cell wall components or dehydrogenase systems, since different microorganisms have been reported to have different dehydrogenase systems (Praveen-Kumar, 2003). This result is also in consonance with the work of Nweke *et al.* (2006).

Table 1. Uninhibited dehydrogenase activities of the bacterial isolates.

Test organisms	**Dehydrogeanase activity (mg formazan/mg cell dry wt/h)**
Escherichia coli	3.017 ± 0.089
Staphylococcus aureus	0.465 ± .0178

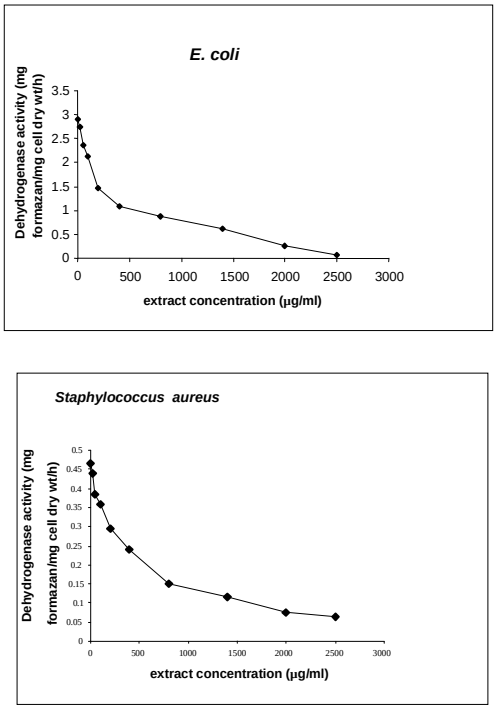

Fig. 1. Dehydrogenase activity in response to various concentrations of leaf extract of *Ocimum gratissimum* by *E. coli* and *S. aureus.*

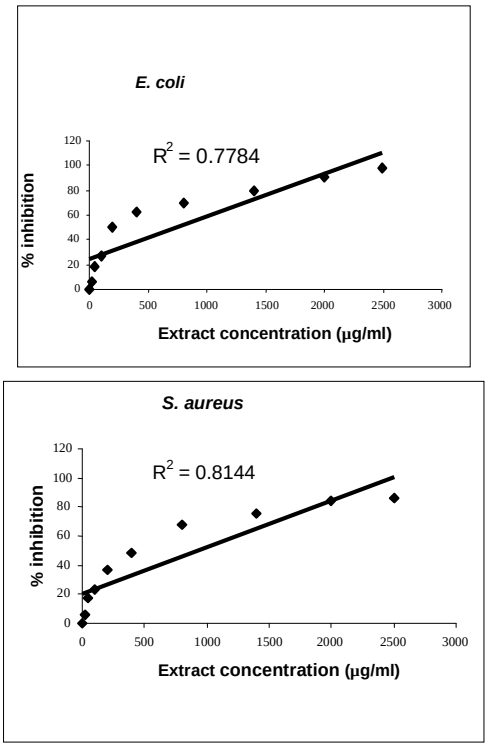

Fig. 2. Extract inhibition of the dehydrogenase activity in *E. coli* and *S. aureus.*

Table 2. Threshold inhibitory concentration of *Ocimum gratissimum* extract against the test Organisms.

Test organisms	**Inhibitory concentrations (µg/ml)**	
	IC_{50}	**IC_{100}**
E. coli	280.19 ± 18.2	2201.75 ± 21.1
Staphylococcus aureus	397.08 ± 23.1	2474.60 ± 40.3

Table 3. *Ocimum gratissimum* inhibition (%) of dehydrogenase in the bacterial isolates.

Test organisms	***Ocimum gratissimum* (µg/ml)**								
	20	**50**	**100**	**200**	**400**	**800**	**1400**	**2000**	**2500**
E. coli	25.38	26.41	28.12	31.54	38.38	52.06	72.58	93.10	100
Staphylococcus aureus	20.96	21.93	23.54	26.76	33.20	46.08	65.40	84.72	100

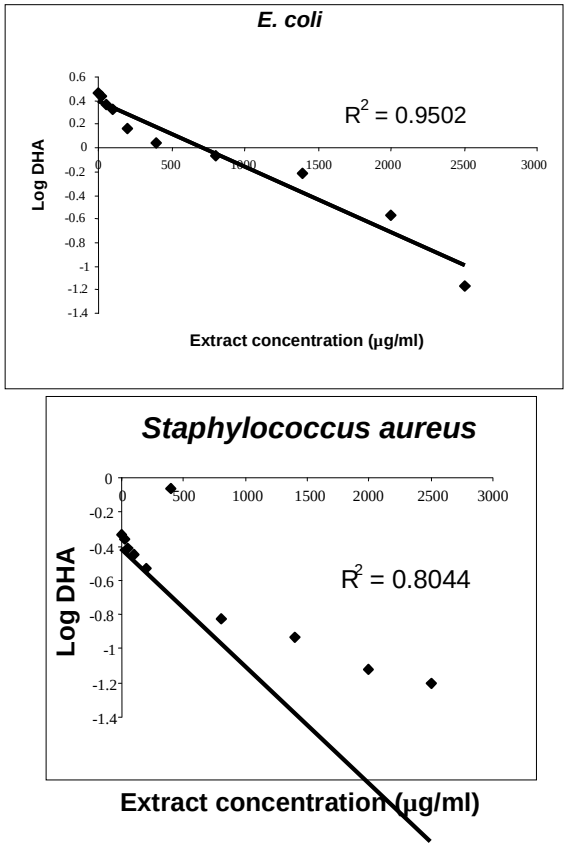

Fig. 3. Correlation of extract concentration with dehydrogenase activity (DHA) in *E. coli* and *Staphylococcus aureus*.

The effects of the different concentrations of the plant extract on the bacterial isolates with respect to the dehydrogenase activity and its inhibition are shown in fig. 1 and fig. 2 and Table 3 The responses of the bacterial dehydrogenase activities to the extracts is concentration-dependent and vary between the organisms. The dehydrogenase activity inhibition observed in this study is consistent with those observed by other workers using plant extracts (Alisi *et al.*, 2008; Nwaogu *et al.*, 2008; Nwaogu *et al.*, 2007). Result presented in fig. 2 showed that *E. coli* had higher percentage inhibition than *S. aureus* at all concentrations of the extract. This implies that *E. coli* was more sensitive to the deleterious effect of the extract of *O.gratissimum* than *S. aureus*.

The dehydrogenase activity (DHA) correlated with the extract concentration with R^2 values greater than 0.80 ($0.8044 \leq R^2 \leq 0.9505$) in both the bacterial strains (Fig. 3). The relationship between the extract concentrations

and the dehydrogenase activity are given as, Log_{10} DHA = -0.0006 extract concentration (µg/ml) + 0.394 and Log_{10} DHA = -0.0004 extract concentration (µg/ml) – 0.03459 for *E. coli* and *S. aureus* respectively. The high R^2 values (>0.80) observed with the two bacterial strains indicated that leaf extract of *O. gratissimum* concentration was a strong determinant of the dehydrogenase activity. This indicated that increase in the extract concentration would have serious deleterious effect on carbon metabolism and respiratory activities of these bacterial strains.The gamma parameter gave good linearization of the dose-response data with R^2 values greater than 0.90 ($0.9098 \leq R^2 \leq 0.9933$) in both bacterial strains (Fig. 4). Table 2 shows the IC_{50} of the leaf extract estimated from the gamma parameter plot at $r^- = 1$ and IC_{100} of the extract estimated from the inhibition data (Fig. 2) for the two bacterial isolates. *E.coli* was more sensitive to the leaf extract than *S. aureus* as depicted by the IC_{50} and IC_{100} of the leaf extract on the bacterial strains. The 2-way ANOVA shows that the dehydrogenase activity varied significantly ($P<0.05$) with bacterial strain and extract concentration.

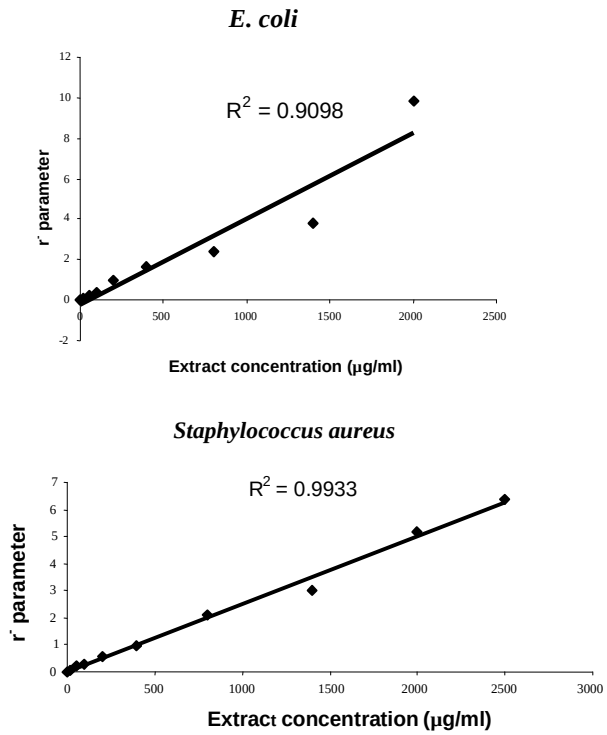

Fig. 4. Linear regression of the gamma parameter (r^-) values obtained from the mean inhibition data of extract against the test organisms.

In conclusion, the extract of *Ocimum gratissimum* inhibited the dehydrogenase activity of *E. coli* and *S. aureus*. The inhibitory action may be due to the presence of different phytochemicals contained in this plant. This further supports the use of leaf extract of *O. gratissimum* as antimicrobial herbal remedies.

References

Adebolu, T.T. and Oladimeji, S.A. (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in Southwestern Nigeria. African Journal of Biotechnology 4(7): 682-684

Akujobi, C.O., Anyanwu, B.N., Onyeze, G.O.C. and Ibekwe, V.I. (2004). Antibacterial activities and preliminary phytochemical screening of four medicinal plants. J.Appl. Sci 7(3): 4328-4338.

Alisi, C.S., Nwanyanwu, C.E., Akujobi, C.O. and Ibegbulem, C.O. (2008). Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extracts *Musa paradisiaca* (Var Sapientum). African Journal of Biotechnology 7(12): 1821-1825.

Aoyama, M. and Nagumo, T. (1997). Effects of heavy metal accumulation in apple orchard soils on microbial biomass and activities. Soil Sci. Plant Nutri. 43: 821-831.

Chander, K. and Brookes, P.C. (1995). Microbial biomass dynamics following addition of metal-enriched sewage sludges to a sandy loam. Soil Biol.Biochem. 27: 1409-1421.

Ezekwesili, C.N., Obiora, K.A. and Ugwu, O.P. (2004). Evaluation of anti-diarrhoeal property of crude aqueous extracts of *Ocimum gratissimum* in rats. Biokemistri 16(2): 122-131.

Gopi, C., Natraja Sekha, Y. and Ponmurugan, P. (2006). In vitro multiplication of *Ocimum gratissimum* through direct regeneration. African Journal of Biotechnology. 5(9): 723-726.

Holetz1, F.B., Nakamura, T.U, Filho, B.P.D., Cortez, D.A.G., Diaz, J.A.M. and Nakamura, C.V. (2003). Effect of essential oil of *Ocimum gratissimum* on *Herpetomonas samuelpessoai*. Acta Protozool. 42: 269-276.

Holt, T.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994). Bergey's manual of determinative bacteriology, 9th ed. Williams, Wilkins. Baltimore .P. 20-32.

Ijaduola, G., Anyiwo, I. and Thomas, C. (1980). *Ocimum gratissimum* and blood coagulation. J. Res. Ethnomed. 1: 19-21.

Kelly, J.J. and Tate, R.L. (1998). Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter. J.Environ.Qual. 27: 609-617.

Kim, C.W., Koopman, B. and Bitton, G. (1994). INT-dehydrogenase activity test for assessing chloride and hydrogen peroxide inhibition of filamentous pure cultures and activated sludge. Water Res. 28: 1117-1121.

Lemos, J.A., Passos, X.S., Fernandes, O.F.L., Paula, J.R., Souza, L.K.H., Lemos, A.A. and Silva, M.R.R. (2005). Antifungal activity of essential oil from *Ocimum gratissimum* towards *Cryptococcus neoformans*. Men.Inst.Oswaldo Cruz. Rio de Janeiro. 100(1): 55-58.

Nakamura, C.V., Nakamura, T.U., Bando, E., Melo, A.F.N., Cortez, D.A.G. and Filho, B.P.D. (1999). Antibacterial activity of *Ocimum gratissimum* essential oil. Men.Inst.Oswaldo Cruz, Rio de Janeiro. 94(5): 675-678.

Nwaogu, L.A., Alisi, C.S., Ibegbulem, C.O. and Igwe, C.U. (2007). Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf. African Journal of Biotechnology 6(7): 890-893.

Nwaogu, L.A., Alisi, C.S., Igwe, C.U. and Ujowundu, C.O. (2008). A comparative study of the antimicrobial properties of ethanolic extracts of *Landolphia owariensis* leaf and root. African Journal Biotechnology 7(4): 368-37217.

Nweke, C.O., Okolo, J.C., Nwanyanwu, C.E. and Alisi, C.S. (2006). Response of planktonic bacteria of New Calabar River to zinc stress. African Journal Biotechnology 5(8): 653-658.

Nweke, C.O., Alisi, C.S., Okolo, J.C. and Nwanyanwu, C.E. (2007). Toxicity of zinc to heterotrophic bacteria from a tropical river sediment.Appl. Ecol. Environ. Res. 5(1): 123-132.

Pino, J.A., Rosado, A. and Fuestes, V. (1996). Composition of essential oil from the leaves of Ocimum gratissimum grown in Cuba. J. Essential Oil Res. 8: 139-141.

Praveen-Kumar, J.C.T. (2003). 2,3,5-triphenyltetrazolium chloride (TTC) as electron acceptor of culturable soil bacteria, fungi and actinomycetes. Biol.Fert.Soils 38: 186-189.

Rogers, E.J. and Li, S.W. (1985). Effects of metals and other inorganic ions on soil microbial activity of water and sedimet communities. World J.Microbiol.Biotechnol. 15: 179-184.

(Received 3 March 2009; accepted 3 October 2009)