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ANTIBACTERIAL ACTIVITY OF *Ocimum gratissimum* LEAVES EXTRACT ON ESCHERICHIA COLI O157

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The aim of this study was to investigate the antibacterial activity of *Ocimum gratis*simum leaves extract against *Escherichia coli* O157. A serial two fold dilution of *Ocimum gratis*simum leaves extract was inoculated with fixed amount ($1*10^7$ cfu) of *E. coli*. The turbidity corresponding to the bacterial growth in various tubes was measured as optical density (O.D.) value at 600nm wavelength with the help of spectrophotometer. The result showed that there is antibacterial activity of the leaves extract against *E. coli* O157. The maximum activity of extract was observed at 1:256 dilution of extract and minimum inhibitory concentration was calculated at 1:512 dilution. All the groups containing extract showed significantly lower colony forming units (C.F.U) as compared to control positive. However, lowest count was observed at 1:256 dilution of 5% extract with maximum percent inhibition of bacterial growth (39.98%) as compared to all other groups. It is conclude that the *Ocimum gratis*simum leaves possess good antibacterial activity and so its growth inhibitory effect against *E. coli* can be used to develop alternative herbal medicines.

Keywords: *Ocimum gratis*simum, leaves extract, *Escherichia coli* 0157

INTRODUCTION

Medicinal plants have been clinically used in curing various human and animal disorders. The whole leaf of *Ocimum gratis*simum contains over 200 compounds including aloesin, anthraquinones (aloin and aloeemodin), acemannan, saponins, sterols, amino acids and vitamins. Most of the compounds have various biological activities with potential health benefits, such as antibacterial, antiviral, wound healing, antioxidant, immunomodulatory, antineoplastic, antihypertensive, antidiabetic and gastroprotective activities (Pandey and Mishra, 2010; Ozsoy et al., 2009; Takzare et al., 2009; Hamman 2008; Chen et al., 2007).
Avian colibacillosis caused by *Escherichia coli* is considered to be the major bacterial disease in the poultry industry world-wide which is associated with heavy economic losses to the poultry industry. *E.coli* strains of serotype 0157 and O2:K1 are often resistant to antimicrobials such as cephradine, tetracyclines, chloramphenicol, sulfonamides, β-lactam antibiotics and amino-glycosides (Li et al., 2007; Rahman et al*.,* 2004). Resistance to fluoroquinolones is also reported in poultry (Li et al*.* 2007; Van den Bogaard et al*.* 2001,). The increasing incidences of diseases have increased the need to develop alternative medicines instead of new antibiotics. Hence the present study was undertaken to examine the *in vitro* activity of *Ocimum gratis*simum against *Escherichia coli* O157, a pathogenic strain of human and animals.

MATERIALS AND METHODS

Preparation of Extracts from *Ocimum gratis*simum leaves

*Ocimum gratis*simum leaves were collected and identified in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. Leaves of *Ocimum gratis*simum were washed, then allowed to dry and aqueous extraction was carried out. The test inoculum was standardized by using the 0.5Mc Farland standard

Determination of Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the compound to inhibit the growth

of microorganisms. The minimum inhibitory concentration values were determined by broth dilution assay and colony forming units. Various dilutions of the extract were prepared. They were inoculated with known amount of *Escherichia coli*.

Broth dilution Assay

Two fold serial dilutions of 5% aqueous extract of *Ocimum grati*ssimum were prepared in sterilized test tubes with sterile normal saline solution upto1:1024. T On the basis of dilution, there were ten groups. Group A to J represented diluted extract from1:2 to 1:1024 dilutions, respectively. Group K contained only normal saline solution (NSS) without the leaves extract, representing control positive whereas Group L contained NSS and (5%) leaves extract and is kept uninoculated representing control negative. The total amount of each dilution was kept 2ml. Each dilution of plant extract was added with equal volume of double strength (2x) of nutrient broth so as to make normal concentration of nutrients after the addition of medium.

All the dilutions of *Ocimum grati*ssimum extract and control tubes (except uninoculated control negative tubes) were inoculated with 0.1 ml of broth culture of test organism containing 1x 10^7 colony forming units (CFU). Following inoculation all the tubes were kept at 37°C and turbidity due to bacterial growth were measured at 600 nm by spectrophotometer as optical density values at 0, 2, 4, 6 and 8 hrs of incubation. The highest dilution of plant extract that showed inhibited growth of test organism as compared with control was considered as MIC (Tsuchia et al., 1996).

Determination of Colony Forming Units

After 8 hrs of incubation a series of 10 fold dilutions of the above mentioned groups was prepared in sterile normal saline solution and 100 µl of the contents was spread evenly on MacConkey's Lactose agar with the help of sterile spreader. The plates were incubated at 37˚ C overnight and the numbers of colonies were counted.
Percent inhibition of bacterial growth in various dilutions of leaves extract with respect to growth in control positive was calculated using the formula (Adedapo et al. 2008): (C.F.U. in control positive -C.F.U. in test group)/C.F.U. in control positive x 100

Statistical analysis

The data was subjected to statistical analysis by applying two way ANOVA using statistical package for social sciences (SPSS) 17^{th} version. Difference between means tested using Tukey (HSD) Post hoc comparisons and significance was set at $P<0.05$.

RESULTS

Mean values of optical density (O.D.) indicating the turbidity due to bacterial growth in various dilutions of *Ocimum grati*ssimum leaves extract and control groups at different incubation periods is shown in Table 1. There was no growth in control negative tubes (Group L). A statistically significant interaction was noticed between the O.D. values of various dilutions of extract at various time intervals of incubation. Group C to H exhibited significantly lower turbidity (bacterial growth) as compared to control and other dilution groups at 2 hours of incubation. At 4 hours of incubation group, OD value was the lowest in group H but did not significantly varied with groups D, E, F and G. However, the OD value in all these groups was significantly lower as compared to control and other groups. The turbidity in group I and J was lower as compared to control but it was non-significant. At 6 hours of incubation, turbidity was lowest in group F but the value did not significantly varied with groups E, G and H. The turbidity in group I was significantly higher as compared to group H but lower as compared to group J and K (control positive). At 8 hours of incubation, group H revealed lowest OD value but the difference between OD value of group F, G, H, and I was non-significant. However, OD values of these groups were significantly lower as compared to control group. The overall means of various dilutions revealed that lowest turbidity was seen in group H. In group I, turbidity was significantly higher as compared to group H and lower as compared to group J and K. Non-significant differences between OD values of group J and K were noticed at various hours of incubation. Thus, the maximum activity of extract was observed at 1:256 dilution (Group H) as the overall turbidity was lowest at this dilution. The MIC was observed at 1:512 dilution (Group I).

Colony forming units

Colony forming units and percent inhibition in bacterial growth in different groups after 8 hours of incubation are given in Table 2. Group L (control negative) revealed no bacterial colony. All the groups containing extract showed significantly lower CFU counts as compared to control positive. However, lowest count was observed in group H (1:256) as compared to all other groups. Group F and G revealed significantly lower CFU as compared to group I, J and K. Group I

and J revealed significantly higher CFU as compared to group H . All the *Ocimum gratis*simum leaves extract containing groups revealed inhibition of bacterial growth with respect to control positive. Maximum percent inhibition of bacterial growth was observed in group H (39.98%) followed by group G, F and E.

DISCUSSION

The results revealed that *Ocimum gratis*simum leaves possessed good antibacterial activity. Although the complete bactericidal effect of extract was not observed in any of the dilutions and even in the undiluted solution but its growth inhibitory effect against *E. coli* was noticed in the present study. Various dilutions of *Ocimum gratis*simum revealed decrease in bacterial growth as compared to control group. Antibacterial activity was not observed at very high as well as very low concentrations of the *Ocimum gratis*simum leaves extracts. The maximum activity of leaves extract was observed at 1:256 dilution of a 5% extract. This suggests that antibacterial activity of *Ocimum gratis*simum occurs at a particular concentration and that higher concentrations are not always preferable. The minimum inhibitory concentration was observed to be 1: 512 dilution of 5% aqueous extract of *Ocimum gratis*simum

Johnson et al. (2011, Li *et al.,* 2007, Prashar et al., 2011) found that *Ocimum gratis*simum had the highest and strong activity against *S. aureus* and *E. coli*. In a study, the *in vitro* antimicrobial activity of *Ocimum gratis*simum extracts was observed as seen by the decreased colony counts in the *Ocimum gratis*simum -treated groups compared with the untreated groups. The extract exhibited excellent antimicrobial activity against *E. coli* and *Vibrio* spp. In results of our study revealed decreased colony forming units and higher inhibition of bacterial growth in extract containing groups as compared to the control. Previous studies concerning the *in vitro* antimicrobial activity of *Ocimum gratis*simum reported that the extracts had antimicrobial activities against both Gram positive and Gram negative bacteria including *S. aureus*, *E. coli*, and *Klebsiella pneumonia*. (Pandey and Mishra, 2010; Ndhlala et al., 2009). Thus, the antibacterial effect of *Ocimum gratis*simum suggest that it has an economical use as a natural antimicrobial supplement.

The overall results from aqueous extracts are encouraging for commercial use. In the light of the fact that microorganism are showing antibiotic resistance against the drugs in use, present investigation is of importance for preparing plant based antimicrobials.

Table 1: Mean optical density (O.D.) values of turbidity due to bacterial growth (*E. coli0157*) at various dilutions of 5% aqueous *Ocimum gratis*simum extract.

5% Aqueous *Ocimum gratis*simum extract dilutions	**Incubation time**					**Overall mean of dilutions**
	0 hr	**2 hr**	**4 hr**	**6 hr**	**8 hr**	
Group A (1:2)	$0.074^{d} \pm .002$	$0.331^{f} \pm .012$	$.443^{e} \pm .003$	$.687^{e} \pm .006$	$.474^{e} \pm .003$	$.402^{e} \pm .003$
Group B (1:4)	$0.065^{a} \pm .001$	$.328^{ef} \pm .017$	$.413^{cde} \pm .009$	$.664^{de} \pm .003$	$.437^{cd} \pm .009$	$.381^{d} \pm .003$
Group C (1:8)	$0.067^{abc} \pm .001$	$.292^{bcdef} \pm .004$	$.422^{de} \pm .019$	$.595^{b} \pm .009$	$.426^{bcd} \pm .003$	$.361^{c} \pm .003$
Group D (1: 16)	$0.068^{abc} \pm .001$	$.278^{bcde} \pm .006$	$.364^{ab} \pm .007$	$.638^{cd} \pm .002$	$.425^{bcd} \pm .003$	$.355^{c} \pm .003$
Group E (1: 32)	$0.068^{abc} \pm .00$	$.274^{bcd} \pm .009$	$.385^{abcd} \pm .003$	$.574^{ab} \pm .007$	$.439^{d} \pm .001$	$.348^{c} \pm .003$
Group F (1: 64)	$0.070^{bcd} \pm .001$	$.254^{ab} \pm .007$	$.371^{abc} \pm .005$	$.556^{a} \pm .003$	$.416^{abc} \pm .003$	$.333^{b} \pm .003$
Group G (1: 128)	$0.072^{cd} \pm .001$	$.255^{ac} \pm .003$	$.355^{a} \pm .003$	$.585^{ab} \pm .003$	$.398^{a} \pm .004$	$.333^{b} \pm .003$
Group H (1: 256)	$0.068^{abc} \pm .001$	$.223^{a} \pm .012$	$.345^{a} \pm .005$	$.561^{a} \pm .007$	$.395^{a} \pm .007$	$.318^{a} \pm .003$
Group I (1: 512)	$0.067^{ab} \pm .00$	$.305^{cdef} \pm .007$	$.407^{bcde} \pm .009$	$.598^{b} \pm .006$	$.413^{ab} \pm .003$	$.358^{c} \pm .003$
Group J (1: 1024)	$0.067^{abc} \pm .001$	$.316^{def} \pm .012$	$.430^{e} \pm .006$	$.632^{c} \pm .006$	$.467^{e} \pm .004$	$.382^{d} \pm .003$
Group K (Control positive)	$0.068^{abc} \pm .00$	$.342^{f} \pm .013$	$.433^{e} \pm .012$	$.647^{cd} \pm .008$	$.484^{e} \pm .003$	$.395^{de} \pm .003$
Group L (Control negative)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 2: Total colony forming units of *E. coli* 0157 in different dilutions of *Ocimum gratis*simum leave extract.

5% Aqueous *Ocimum gratis*simum root extract dilutions	CFU/100µl after 8 hrs	Percent Inhibition w.r.t. control positive
Group A (1:2)	$23.3^{g} \pm 1.53 \times 10^{7}$	5.89
Group B (1:4)	$22.76^{fg} \pm 1.20 \times 10^{7}$	8.08
Group C (1:8)	$22.1^{ef} \pm 2.08 \times 10^{7}$	10.74
Group D (1:16)	$20.2^{d} \pm 1.15 \times 10^{7}$	18.42
Group E (1:32)	$18.56^{c} \pm 3.48 \times 10^{7}$	25.04
Group F (1:64)	$17.53^{bc} \pm 2.60 \times 10^{7}$	29.2
Group G (1:128)	$17.03^{b} \pm 2.03 \times 10^{7}$	31.22
Group H (1:256)	$14.86^{a} \pm 1.76 \times 10^{7}$	39.98
Group I (1:512)	$21.03^{de} \pm 0.88 \times 10^{7}$	15.06
Group J (1:1024)	$23.40^{g} \pm 2.31 \times 10^{7}$	6.49
Group K (Control positive)	$24.76^{h} \pm 3.38 \times 10^{7}$	0
Group L (Control negative)	0.00 ± 0.00	-

Mean ± S.E. with unlike superscript in the column differ significantly ($P < 0.05$).

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