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Research Article

INVESTIGATION OF *IN VITRO* ANTIBACTERIAL ACTIVITY OF *ALOE VERA* LEAVES EXTRACT ON *ESCHERICHIA COLI*

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ABSTRACT

The present study was undertaken to investigate the antibacterial activity of *Aloe vera* leaves gel extract against *Escherichia coli* O78. In serial two fold dilution of *A. vera* leaves aqueous extract, $1x10^7$ colony forming units (CFU) of *E. coli* were added. The turbidity corresponding to the bacterial growth in different dilutions was measured as optical density at 600 nm with a spectrophotometer. The maximum activity of extract was observed at its 1:256 dilution and minimum inhibitory concentration was found to be 1:512 dilution. All the dilutions showed significantly lower CFU as compared to control positive. However, the lowest count was observed at 1:256 dilution of 5% extract with maximum percent inhibition of bacterial growth (39.9%) as compared to all other dilutions (1:2 to 1:1024). The gel of *A. vera* leaves possessed antibacterial activity against *E. coli* and suggested its possible use as a herbal medicine.

Keywords: *Aloe vera*, leaves extract, *Escherichia coli*, *In vitro*

Medicinal plants have been clinically used in curing various human and animal disorders. *Aloe vera*, is a spiky, succulent and perennial plant. The whole leaf of *A. vera* contains over 200 compounds and these compounds have antibacterial, antiviral, wound healing, antioxidant, immunomodulatory, antineoplastic, antihypertensive, antidiabetic and gastroprotective activities (Hamman, 2008; Takzare *et al*., 2009). The leaf of *A. vera* can be divided into gel and peel.

Avian colibacillosis caused by *Escherichia coli* is one of the major bacterial diseases of poultry. Different serotypes of *E. coli* have been reported to be resistant to different antimicrobials (Rahman *et al.*, 2004; Li *et al*., 2007). The increasing incidences of infectious 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 *Aloe vera* against *E. coli* O78 a pathogenic strain of poultry.

MATERIALS AND METHODS

Preparation of Extracts from Aloe vera Leaves: *Aloe vera* leaves were collected from Medicinal and Aromatic Plants Section, Department of Genetics and Plant Breeding, College of Agriculture, CCSHAU, Hisar. The leaves were washed and pulp/gel was taken out after removing the outer covering. For aqueous extraction, the pulp was sliced in small pieces and then blended for 3 min (Githiori *et al*., 2003). The blended material was squeezed through a muslin cloth. Then 5 gm of the gel was dissolved in 100 ml of distilled water so to make 5% aqueous solution which was stored at 4^0C till further use.

Determination of Minimum Inhibitory Concentration (MIC): The MIC values were determined by broth dilution assay and colony forming units (CFU). Further dilutions of 5% aqueous extract were prepared and were inoculated with known amount of *E. coli*. The *E. coli* O78 strain, isolated from a natural case of colibacillosis in poultry, was obtained from the Department of Veterinary Public Health and Epidemiology of this university.

Broth Dilution Assay: Two fold serial dilutions (upto1:1024) of 5% aqueous extract were prepared in normal saline solution (NSS) which formed ten groups: Groups A to J represented diluted extract from 1:2 to 1:1024, respectively. Group K (positive control) contained only NSS without the extract whereas Group L (negative control) contained NSS and 5% leaves extract and was kept uninoculated. The total amount of each dilution used was 2ml. To each dilution, equal volume of double strength (2x) nutrient broth was added so as to make normal concentration (1x) of nutrients after the addition of medium.

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All the dilutions of *A. vera* extract and positive control tube (except group L) were inoculated with 0.1 ml of broth culture of *E. coli* containing $1x10^7$ CFU. Following inoculation, all the tubes were kept at 37°C and turbidity due to bacterial growth was measured at 600nm by a spectrophotometer as optical density (O.D.) values at 0, 2, 4, 6 and 8 h of incubation. The highest dilution of plant extract that inhibited growth of test organism as compared with control was considered as MIC (Tsuchia *et al*., 1996).

Determination of CFU: After 8 h of incubation, 10 fold dilutions of the above mentioned groups were prepared in sterile NSS and 100 µl of the contents were spread evenly on MacConkey's Lactose agar. The plates were incubated at 37^0C overnight and the number of colonies was counted.

Percent inhibition of bacterial growth in various dilutions of leaves extract with respect to growth in positive control was calculated using the formula (Adedapo *et al*. 2008)= (CFU in positive control -CFU in test group)/CFU in positive control x 100

Statistical Analysis: The data were subjected to two way ANOVA using Statistical Package for Social Sciences (SPSS) 17th version. Difference between means tested using Tukey (HSD) Post hoc comparisons and significance was set at $P<0.05$.

RESULTS AND DISCUSSION

There was no growth in group L (Table 1). A statistically significant interaction was noticed between the O.D. values of various dilutions of extract at various time intervals of incubation. Groups C to H exhibited significantly lower turbidity (bacterial growth) as compared to positive control and other dilution groups (A, B, I and J) at 2 h of incubation (Table 1). At 4 h of incubation (Table 1), O.D. value was the lowest in group H but did not differ significantly from groups D, E, F and G. However, the O.D. value in all these groups (D, E, F, G and H) was significantly lower as compared to positive control and other groups (Table 1). The turbidity in groups I and J was lower as compared to positive control at 4 h of incubation.

At 6 h of incubation, turbidity was the lowest in group F but the value did not differ significantly from groups E, G and H. The turbidity in group I was significantly higher as compared to group H but lower as compared to groups J and K. At 8 h, group H revealed the lowest O.D. value but the difference between OD value of groups F, G, H, and I was non-significant. However, O.D. values of these groups were significantly lower as compared to group K. The overall means of various dilutions revealed that the lowest turbidity was seen in group H. In group I, turbidity was significantly higher as compared to group H and lower as compared to groups 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 the lowest at this dilution. The MIC was observed at 1:512 dilution (group I).

Colony Forming Units: Group L revealed no bacterial growth. All the groups containing extract showed significantly lower CFU counts as compared to group K (Table 2). However, the lowest count was observed

Table 1
Mean optical density (O.D.) values of turbidity due to *E. coli* at different dilutions of 5% aqueous *A. vera* extract

5% aqueous *A. vera* extract dilutions	Incubation time (h)					Overall mean of dilutions
	0	2	4	6	8	
Group A (1:2)	0.074^{d}±0.00	0.331^{f}±0.01	0.443^{e}±0.00	0.687^{e}±0.01	0.474^{e}±0.00	0.402^{e}±0.00
Group B (1:4)	0.065^{a}±0.00	0.328^{ef}±0.02	0.413^{cde}±0.01	0.664^{de}±0.00	0.437^{cd}±0.01	0.381^{d}±0.00
Group C (1:8)	0.067^{abc}±0.00	0.292^{bcdef}±0.00	0.422^{de}±0.02	0.595^{b}±0.01	0.426^{bcd}±0.00	0.361^{c}±0.00
Group D (1: 16)	0.068^{abc}±0.00	0.278^{bcde}±0.01	0.364^{ab}±0.01	0.638^{cd}±0.00	0.425^{bcd}±0.00	0.355^{c}±0.00
Group E (1: 32)	0.068^{abc}±0.00	0.274^{bcd}±0.01	0.385^{abcd}±0.00	0.574^{ab}±0.01	0.439^{d}±0.00	0.348^{c}±0.00
Group F (1: 64)	0.070^{bcd}±0.00	0.254^{ab}±0.01	0.371^{abc}±0.01	0.556^{a}±0.00	0.416^{abc}±0.00	0.333^{b}±0.00
Group G (1: 128)	0.072^{cd}±0.00	0.255^{ac}±0.00	0.355^{a}±0.00	0.585^{ab}±0.00	0.398^{a}±0.00	0.333^{b}±0.00
Group H (1: 256)	0.068^{abc}±0.00	0.223^{a}±0.01	0.345^{a}±0.01	0.561^{a}±0.01	0.395^{a}±0.01	0.318^{a}±0.00
Group I (1: 512)	0.067^{ab}±0.00	0.305^{cdef}±0.01	0.407^{bcde}±0.01	0.598^{b}±0.01	0.413^{ab}±0.00	0.358^{c}±0.00
Group J (1: 1024)	0.067^{abc}±0.00	0.316^{def}±0.01	0.430^{e}±0.01	0.632^{c}±0.01	0.467^{e}±0.00	0.382^{d}±0.00
Group K (Control positive)	0.068^{abc}±0.00	0.342^{f}±0.01	0.433^{e}±0.01	0.647^{cd}±0.01	0.484^{e}±0.00	$0.395d^{e}$±0.00
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

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

Table 2
Total colony forming units of E. coli in different dilutions ofA. veraleaves extract

5% aqueous *A. vera* extract dilutions	CFU/100µl after 24 h of incubation ($x10^7$)	Percent inhibition w.r.t. positive control
Group A (1:2)	$23.3^{g}\pm1.53$	5.89
Group B (1:4)	$22.76^{fg}\pm1.20$	8.08
Group C (1:8)	$22.1^{ef}\pm2.08$	10.74
Group D (1:16)	$20.2^{d}\pm1.15$	18.42
Group E (1:32)	$18.56^{c}\pm3.48$	25.04
Group F (1:64)	$17.53^{bc}\pm2.60$	29.2
Group G (1:128)	$17.03^{b}\pm2.03$	31.22
Group H (1:256)	$14.86^{a}\pm1.76$	39.98
Group I (1:512)	$21.03^{de}\pm0.88$	15.06
Group J (1:1024)	$23.40^{g}\pm2.31$	6.49
Group K (Positive control)	$24.76^{h}\pm3.38$	0
Group L (Negative control)	0.00±0.00	-

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

in group H as compared to all other groups. Groups F and G revealed significantly lower CFU as compared to groups I, J and K (Table 2). Groups I and J revealed significantly higher CFU as compared to group H. All the *A. vera* leaves extract containing groups revealed inhibition of bacterial growth when compared to control positive. Maximum percent inhibition of bacterial growth was observed in group H (39.98%) followed by groups G, F and E (Table 2).

The results revealed that *A. vera* leaves possessed antibacterial activity. Maximum activity of *A. vera* leaves extract was observed at 1:256 dilution of the 5% extract. Prashar *et al*. (2011) also observed anti *E. coli* activity of aloe species extract. Although the complete bactericidal effect of aloe extract was not observed in any of the dilutions and even in the undiluted solution but growth inhibitory effect of *A. vera* against *E. coli* was noticed in the present study. Antibacterial activity was not observed at very high as well as at very low concentrations of the aqueous *A. vera* extract. Darabighane *et al*. (2011) reported that broilers treated with 2% *A. vera* gel showed more performance in terms of body weight gain, feed intake and ileum morphology as compared to those treated with 1.5 and 2.5% *A. vera* gel.

Previous studies had revealed that aloe extracts had antimicrobial activities against both Gram positive and Gram negative bacteria including *S. aureus*, *E. coli*, vibrio and *Klebsiella pneumoniae in vitro* (Pandey and Mishra, 2010; Kwon *et al*., 2011). Tian *et al*. (2003) reported that aloe-emodin and aloin changed the morphology of *E. coli* and damaged its outer cell structure. The overall results from aqueous extracts of *A. vera* are encouraging for commercial use. In the light of increased antibiotic resistance, the present investigation is of importance for preparing *A. vera* based antimicrobials.

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