ISSN 0974 – 5211

Journal of Natural Products

Volume 6 (2013)

www.JournalofNaturalProducts.Com

Research Paper

Effect of Guava (*Psidium guajava*) leaf meal on production performances and antimicrobial sensitivity in commercial broiler.

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(Received 29 June 2013; Revised 02 July- 04 August 2013; Accepted 06 August 2013)

ABSTRACT

The study was conducted at the poultry shed and poultry laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh in order to apply the possibility of treated guava (*Psidium guajava*) leaf meal in broiler diets to determine the effects of it's at various levels of dietary treatment on production and quality characteristics of broiler. For this 180 day old broiler chicks (Cobb 500) were taken and divided into four treatments, each with three replications (15 birds/ replication) at the age of four or day and then offered manually prepared diets supplemented with 0%, 2.5%, 3.5%, 4.5% guava leaf meal after treating by means of some physical and chemical processes. The result showed that, feed intake, body weight gain and feed conversion ratio at different dietary treatments were almost similar and the differences were statistically non-significant except fat content, mortality rate and antimicrobial sensitivity. Fat content and mortality rate were decreased with increased level of guava leaf meal up to 4.5% level. However guava leaf extract had significant effect on antibacterial activity basically higher against *E. coli* followed by *streptococcus* sp. and *staphylococcus* sp. and was significant at 5 % level of significance. Based on the results of present study it may be concluded that guava leaf is a good source of nutrients and it has significant effect on fat content of broiler, mortality rate and antimicrobial sensitivity without affecting the bird's feed intake, body weight and feed conversion ratio. The results of the study suggest that supplementation of guava leaf meal (*Psidium guajava*) up to 4.5% level in diets has high potential as commercial applications for production performance and antimicrobial sensitivity of broiler.

Keywords: Guava leaf; Broiler; Antimicrobial sensitivity.

INTRODUCTION

Meat and other animal products can play a significant role in alleviating the nutritional status of the people. Meat is an excellent source of high quality and readily

digestible protein. They are also good sources of micronutrients (Bender, 1992). Over the last century, the amount and proportion of animal fat in human diets has increased in many societies.

The biggest impediments to livestock production in developing Bangladesh are the high cost of feed ingredients. Feed additives have also been widely used in poultry industry since long time as tool to increase animals' performance in regard to growth and feed efficiency (Collington, et al., 1990). Therefore, about 80% of domestic animals have been fed synthetic compounds for the purpose of either medication or growth promotion (Lee, et al., 2001). However, due to growing concerns about antibiotic resistance and the potential for a ban for antibiotic growth promoters in many countries in the world, there is an increasing interest in finding alternatives to antibiotics in poultry production (Ashayerizadeh, et al., 2011; Cardozo, et al., 2004; Tipu, et al., 2006). Under the intensive management systems, herbal extracts are already being used as feed supplements to improve growth performance (William and Losa, 2001). These extracts when supplemented to animal's diets can play a role in supporting both performance and health status of the animal (Horton, et al., 1991; Bakhiet and Adam, 1995; Skrabka Blotnicka, et al., 1997; Gill, 2000; Manzanilla, et al., 2001) along with controlling the population of harmful intestinal microflora. Unfortunately, nearly all sources of agricultural leaf and plant protein posses associated high fiber and anti-nutritional factors which must be eliminated by special processing techniques to make them of maximum nutritional value. A great quantity of guava leaf meal (pulp and peel) is produced as a waste of canning industry in Egypt and yet was not fully evaluated as a feedstuff for poultry. Aly et al., (1981) found that guava leaf contained 8.9% oil. The protein content of guava leaf was 9.73% on dry weight consumed fresh and also processed (beverages, syrup, ice cream, and jams). Pulp and peel fractions were tested, and both showed high content of dietary fiber (48.55-49.42%) and extractable polyphenols (2.62-7.79%). These results indicate that guava could be a suitable source of natural antioxidants. Peel and pulp could also be used to obtain antioxidant dietary fiber.

Again guava is commonly known as the poor man's apple of the tropics has a long history of traditional use, much of which is being validated by scientific research. Guava is rich in tannins, phenols, flavanoids, essential oils, lectins, vitamins, fatty acids etc (Geidam, et al., 2007). Much of the guava's medicinal activity is attributed to these flavanoids. The flavanoids have demonstrated anti-bacterial activity.

Plants are used in treating malaria, diarrhoea, burns, gonorrhoea, stomach disorders and other infectious diseases. Tremendous efforts of scientists have been employed in establishing plants with promising antimicrobial activity and yielding fruitful results (Adedayo, et al., 2001; Ndukwe, et al., 2005; Aibinu, et al., 2007). Extracts of roots, bark and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothaches, coughs and a number of other conditions (Morton, 1981). In present work we observe the effect of guava leaf on production performances and quality characteristics of broiler and to establish the antibacterial effect of guava leaf on broiler.

MATERIALS AND METHODS

Source of Guava (Psidium guajava) leaf: Guava leaf was collected from different places of Dinajpur district of Bangladesh. The leaves were coarsely powdered after

treating by means of physical and chemical processes. Then it was directly mixed with manually prepared diets in 0%, 2.5%, 3.5% and 4.5% doses, accordingly.

Treatment of guava leaf as feed samples: At first guava leaf was boiled in water for one hour, boiled in alkaline solution 0.1 N for one hour. Then alkali treated guava leaf was boiled in acid solution 0.1 N for one hour, and autoclaved for 20 minutes at 15 IP pressure. Chemical analysis was conducted on both raw and treated guava leaves. Treated guava leaves were dried at 80°C in an electric oven and grind in hummer mill then samples were taken for determination of chemical composition according to AOAC (1990).

Experimental diets: The experimental diets in mash form and drinking water was provided *adlibitum.* All diets were formulated manually to meet the nutrient requirements of broiler (NRC, 1994) .The chicks were fed starter diet from 1 to 10 days, grower diet from 11-20 days and a finisher diet from 21 to 42 days old broiler. Basically Tables (1, 2 and 3) show the composition and the chemical composition of the starter, grower and finisher rations, respectively. The experimental diets were designed as-

T_1 : control
T_2 : control+ 2.5% guava leaves as mash form
T_3 : control+ 3.5% guava leaves as mash form
T_4 : control + 4.5% guava leaves as mash form

Experimental design: The experiment was conducted at the open sided poultry shed in Hajee Mohammad Danesh Science and Technology University, Dinajpur. A total 180 day-old broiler chick (Cobb 500) were purchased from CP Bangladesh Ltd. All animal experiments were carried out according to the guidelines of "Cobb 500 Breeder Management Guide". At first chicks were reared at brooding house to adjust with the environmental condition up to 10 days. After 10 days chicks were randomly assigned to their treatments and was divided into four dietary treatment groups composed of 45 chicks in each; each treatment was composed of three replications with 15 birds in each in a complete randomized design (CRD).

Bird's management*:* The birds were housed on floor and routinely managed as any other commercial broiler flock. Heating was provided by a single electric brooder, where the initial temperature was set at 37°C and decreased by 1°C per day to final temperature of 28°C at the end of experiment. Supplementary heating was provided as required by mobile butane gas heaters besides to electricity heater. During brooding period, linear feeder and round plastic drinker were used. After that linear feeder was replaced by round plastic drinker. Feed and fresh water were offered to the bird manually according to experimental schedule. One round plastic feeder and drinker were provided for seven birds**.** All birds were vaccinated against Newcastle disease at day one and boostering by day 21. Against Gumboro disease the birds were vaccinated firstly at day seven and boostering at day 14. At very first week Gluco- C was used @ 50g/liter water. Water solublable vitamin Rena WS @ 1g/liter and normal saline also provided for first 3 days of brooding.

Observation of birds*:* All the birds were examined twice daily for any visible physical changes like restlessness, lordosis, abnormal gait, vices and depression as well as feeding style during study period.

The performance trial*:* During the 42 days of experimental period, growth performance was evaluated. Before treatment, body weight was taken for each group of birds. Then body weight and feed consumption were recorded daily and body gain and feed conversion ratio were then calculated by using the following formula. Mortality was recorded throughout the study period.

Feed intake =Feed intake in replication/No. of birds in replication.
Body weight gain (kg) = Final weight – Initial weight.
FCR = Feed intake (kg)/Weight gain (kg)

Determination of Antimicrobial activity of guava leaf extract

Collection of samples*:* Swaps were taken using sterile swap sticks from the naval of day-old chicks. These were inoculated into the plates containing MacConkey and blood agars. Using the half and quarter plate streaking method, respectively.

Culturing and identification of the organisms*:* The inoculated plates were incubated immediately for 24h at 37°C. The growth was then identified using colonial appearance, gram stain, examination of the organisms under microscopes. Sub-culture was done by using different media for confirmation of the organisms earlier identified. All media used were prepared according to manufacturer's instructions.

Determination of Antimicrobial properties

Bacterial isolates: The bacteria were isolated by special media culture; e.g. MacConkey for *E. coli.* The bacterial isolates from the naval of day-old-chicks were used for determining the anti bacterial properties of guava leaf extract. The isolates were propagated and stored on nutrient agar plates. All the isolates were maintained on nutrient agar plate at 4°C and sub-cultured in nutrient broth at 37°C for 8 hours prior to antimicrobial testing. One milliliter of the broth culture was then used to flood the agar plates.

Concentration of extracts*:* Stock solutions of the extract were prepared by dissolving known weight of the extract in known volume of distilled water 0.01, 0.02 and 0.04g of the extracts were dissolved in 1ml of distilled water to afford 100, 200 and 400mg/ml of the extract, respectively. Standard antibacterial agent oxytetracycline (Renamycin -500mg, renata animal health. Bangladesh) at a concentration of 10mg/ml was also used on all the bacteria and the zones of inhibition compared with those of the plant extract.

Antibacterial sensitivity testing*:* Bauer-Kirby disc diffusion method as described by Bauer et al. (1966) was used to determine the antibacterial activity. Discs containing different concentrations of dissolved extract were prepared. Sterilized filter papers (Whatman No. 1, 6mm in diameter) soaked in beakers containing different concentrations (100, 200 and 400mg/ml) of the extract. Overnight cultures of each bacterial isolate were spread on the surface of dried nutrient agar plates. The plates were incubated at 37°C for 30 min before the discs were applied aseptically. The treated plates were incubated at 37°C for 48 hours. The same procedure was carried out with the oxytetracycline (10mg/ml) as standard antibiotic. Plates without the antibiotic or extract discs were set up as control experiment. The zones of inhibition above 6mm diameter of each isolate were used as measure of susceptibility to the extracts and were compared to that of the standard antibiotic.

Determination of minimum inhibitory concentration (MIC) of the extracts*:* The MIC was determined using the method described by Greenwood (1989). For each extract three sterile test tubes were arranged in a test tube rack in a row for each organisms and 0.5ml of sterile nutrient broth was pipetted into each tube. Half a millimeter of the crude extract containing 100mg/ml was pipetted into tube one to obtain a concentration of 50mg/ml. There after there was a serial dilution of the extract to obtain concentrations of 25, 12.5, 6.25 and 3.13mg /ml, respectively. 0.5ml of the test organism was pipetted into each test tube and incubated at 37°C for 24 hours. The MIC was recorded as the least concentration of plant extract that completely inhibit the growth of the test organism.

Statistical analyses: Data were analyzed by two factor analysis (diet and strain) of variance using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986). The significance differences between the treatment means were calculated by the Duncan's Multiple Range Test. All analyses were performed by MSTATC and SPSS Program.

RESULTS AND DISCUSSION

Effect of guava leaf meal on body weight gain*:* Body weight gain in different dietary treatments during experimental periods was almost similar and the differences were not significant (*P>0.05*) (Table 4). These results indicate that inclusion up to 4.5 percent guava leaf meal had no adverse effect on body weight gain. This result similar with El-Deek et al. (2009) study. They had found that the final results of broiler body weight and body weight gain at 8 wks of age showed no significant differences as the result of feeding 2 or 4% levels of guava by-products, raw or treated, in comparison with the control. Moreover, feeding with the higher levels of raw or treated samples 6 to 8% showed slightly reduction of broiler body weight and body weight gain, but not significantly. They also added that this observation could be due to the presence of higher amount of fiber compared to the other treatments.

Effect of guava leaf meal on feed intake*:* Feed intake of broilers in different dietary treatments during experimental periods was almost statistically similar and the differences were non-significant (*P>0.05*) (Table 4). So, the result clearly showed that guava leaf meal up to 4.5 percent dietary level had no detrimental effect on feed consumption. The result supported by Abiola and Adekunle (2002). They had found that high fiber diets increased feed intake. If the chickens fed diet include the autoclaved sample and sample treated with alkaline will consume more digestible fiber than those fed the raw or the other ones. Gadalla (1983) arrived to similar finding with autoclaving apricot kernel meal. Abiola et al., (2002) reported that the alkali treatment of melon husk increased the feed intake with increase in the level of alkali treatment of melon husk in the diet.

Effect of guava leaf meal on feed conversion ratio: Feed conversion ratio in different dietary treatments at 2.5, 3.5 and 4.5 percent level was almost similar and the differences were non-significant (*P>0.05*) (Table 4).The results indicate that there was no detrimental effect on feed conversion ratio after feeding up to 4.5 percent level of guava leaf meal. Similar result was found by El-Deek et al., (2009). They reported that the broiler given diet with 2 or 4% guava by-products utilized their diets more efficiently than those fed on diets with 6 or 8% during the finishing period.

Effect of guava leaf meal on fat content*:* This study showed that fat content of broiler was decreased significantly by supplementation of guava leaf meal in broiler-ration (*P>0.05).* It is evident from Table 5 that the tendency of reduced fat content was observed in the dietary treatments with inclusion of 2.5-4.5% guava leaf meal. Although this result is dissimilar with El-Deek et al., (2009) because they had found that the abdominal fat weight showed no significant differences in the relative weight for the broiler received 2, 4 or 6% raw or treated by-products. But, broiler receiving 8% raw or treated guava by-product have significantly less abdominal fat than any other dietary level or the control.

Effect of guava leaf meal on mortality rate: In this experiment, the experimental diets produced a decrease mortality rate in comparison to control. The reduced mortality rate was obtained at 4.5% level of guava leaf meal supplementation (Table 5). The results remarkably differ with the El-Deek et al., (2009). They had found that

the processing technique of guava leaf had no effect on mortality rate, regardless of the inclusion levels. However, when the percentages of guava by-products inclusion in the diets increased to 6 and 8%, a significant increase in mortality rate was evident, regardless of the processing employed.

Antimicrobial activity of guava leaf*:* Two hundred and fifty grams of the dried powder leaves of guava was exhaustively extracted with 1.5L of distilled water in a reflux apparatus and then concentrated to yield 70.8g of the crude extracted that is 28.3% w/w with respect to the dried powdered extract. Three different bacterial organisms were isolated from the 60 swabs taken from the navel of broilers day-old-chicks. The isolated organisms include; *Staphylococcus* sp., *E. coli Streptococcus* sp. All these three organisms were isolated from broiler day-old-chicks while only four of the organisms (*Staphylococcus* sp., *Streptococcus* sp., *E. coli*.) were isolated from the broiler day-old-chicks (Table 6). The effect of the three different concentrations of the extract on the bacteria isolated is presented in Table 7. The extract showed concentration dependent antibacterial activity against *E. coli, Streptococcus* sp., *Staphylococcus* sp.

A study of the antibacterial effect of guava aqueous leaf extract on bacterial organisms isolated from the navel of day old chicks was carried out. The result of the study showed that guava leaf extract have concentration dependant inhibitory effect on the growth of *E. coli, Staphylococcus* sp., *Streptococcus* sp. isolated from the navel of day-old chicks (Table 8). Similar results on growth inhibition were obtained by Gnan and Demello (1999), when testing the effect of the extract on *Staphylococcus aureus* by using guava leaf water extract.Iwu (1993) reported antibacterial effect guava leaf extract against *E. coli, Staphylococcus aureus, Streptococcus.* All the bacteria inhibited by the leaf extract have been incriminated in omphalitis as shown by Jordon and Pattison (1999).

The susceptibility test of the extract (400mg/ml) against most of the organisms screened indicated that *E. coli* exhibited the highest inhibition zone of 25 mm which could be compared favourably with 30 mm of Oxytetracycline (20mg/ml). The activity of the extract against *E. coli* is important since many avian pathogenic *E. coli* strains have been reported to be resistant to common antibacterial agents used in poultry production (Ewers et al., 2003).The minimum inhibitory concentration against the susceptible organisms indicated that *E. coli* had the lowest, suggesting that the extract can be a potential antibacterial agent if the active compound responsible is isolated.

Phytochemical evaluation of the leaf has shown the presence of flavonoids, tannins, saponins, Phenols lectins, triterpenes and carotenoids (Geidam, et al., 2007). These compounds are known to be biologically active. The antimicrobial activity of the leaf extracts demonstrated can be attributed to the presence of flavonoids (Ali and Shamsuzzaman, 1996). Similarly, Berdy et al., (1981) demonstrated that the antibacterial effect could also be due to guajaverine and psydiolic acid, which are also present in the leaf. Flavonoids derivatives have been found to inhibit the growth of *Staphylococcus aureus* at the dilution of 1: 10,000 (Ali, et al., 1996). This is medically important in the treatment of inflamed tissues and lectins in guava were shown to bind to *E. coli* preventing its adhesion to the intestinal wall and thus preventing infection (Berdy, et al., 1981). Therefore, the activity of the extract against the isolated organisms in this study could be linked to the aforementioned reports. These effects can explain the long history of guava use in traditional medicine as a cure for many bacterial diseases. In a word, this study has provided a basis for the use

of *Psidium guajava* in the treatment of yolk sac infection caused by *E. coli*, *Staphylococcus* sp. and *Streptococcus* and either primarily or in combination.

CONCLUSION

It may be concluded that guava leaf (*Psidium guajava*) is a good source of nutrients and it has significant effect on fat content of broiler, mortality rate and antimicrobial sensitivity without affecting the bird's feed intake, body weight and feed conversion ratio.

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Table - 1: Composition of the experimental starter diets fed to broilers.

Feed ingredients	Dietary level of guava leaves			
	T_1 (0%)	T_2 (2.5 %)	T_3 (3.5 %)	T_4 (4.5 %)
Maize	49.60	49.60	49.20	49.00
Soybean meal	26.90	25.04	24.85	24.50
Rice polish	10.90	10.77	10.70	10.00
Meat & bone meal	8.00	8.00	8.00	8.00
DCP	0.71	0.70	0.70	0.70
Soybean oil	3.50	3.00	2.73	2.88
Guava leaves	0.00	2.50	3.50	4.50
Salt	0.27	0.27	0.30	0.30
Vitamin-mineral premix*	0.12	0.12	0.12	0.12
Elements	**Calculated composition**			
ME (Kcal/Kg)	3084	3106.5	3124.5	3133
CP (%)	21.40	21.35	21.30	21.28
CF (%)	3.77	3.71	3.78	3.78
Ca (%)	1.16	1.12	1.12	1.13
P (%)	0.54	0.54	0.55	0.55
Lysine (%)	1.19	1.19	1.18	1.18
Methionine (%)	0.48	0.48	0.48	0.48

Table - 2: Composition of the experimental grower diets fed to broilers.

Feed ingredients	Dietary level of guava leaves			
	T_1 (0%)	T_2 (2.5%)	T_3 (3.5 %)	T_4 (4.5 %)
Maize	52.00	52.00	52.00	52.00
Soybean meal	22.78	22.13	21.13	20.13
Rice polish	12.70	11.70	11.70	11.70
Meat & bone meal	8.00	7.00	7.00	7.00
Soybean oil	3.50	3.70	3.70	3.70
DCP	0.75	0.70	0.70	0.70
Guava leaves	0.00	2.50	3.50	4.50
Salt	0.27	0.27	0.27	0.27
Vitamin-mineral premix*	*	*	*	*
Elements	**Calculated composition**			
ME (Kcal/Kg)	3120	3142.5	3130.5	3169
CP (%)	18.85	18.76	18.69	18.85
CF (%)	3.69	3.70	3.71	3.69
Ca (%)	1.06	1.07	1.07	1.06
P (%)	0.51	0.50	0.52	0.51
Lysine (%)	1.01	1.00	1.00	1.01
Methionine (%)	0.40	0.40	0.40	0.40

Table - 3: Composition of the experimental finisher diets fed to broilers.

Feed ingredients	Dietary level of guava leaves			
	T_1 (0%)	T_2 (2.5 %)	T_3 (3.5 %)	T_4 (4.5%)
Maize	55.30	55.00	55.00	55.00
Soybean meal	22.53	21.83	21.00	20.33
Rice polish	10.70	10.70	10.70	10.70
Meat & bone meal	7.00	6.00	6.00	6.00
DCP	0.70	0.70	0.70	0.70
Soybean oil	3.50	3.00	2.74	2.50
Guava leaves	0.00	2.50	3.50	4.50
Salt	0.27	0.27	0.27	0.27
Vitamin-mineral premix*	*	*	*	*
Elements	**Calculated composition**			
ME (Kcal/Kg)	3120	3142.5	3130.5	3169
CP (%)	18.85	18.76	18.69	18.85
CF (%)	3.69	3.70	3.71	3.69
Ca (%)	1.06	1.07	1.07	1.06
P (%)	0.51	0.50	0.52	0.51
Lysine (%)	1.01	1.00	1.00	1.01
Methionine (%)	0.40	0.40	0.40	0.40

- Broiler premix was added @ 120 g per 100 kg which contained: vitamin A: 4800 IU; vitamin D: 960 IU; vitamin E: 9.2 mg; vitamin k_3: 800 mg; vitamin B_1: 600 mg; vitamin B_2: 2 mg; vitamin B_3: 12 mg; vitamin B_5: 3.2 mg; vitamin B_6: 1.8 mg; vitamin B_9: 2 mg; vitamin B_{12}: 0.004 mg; Co: 0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; I: 0.6 mg; Mn: 19.2 mg; Zn: 16 mg; Se: 0.48 mg; DL – Methionine: 20 mg; L-lysine:12 mg.

Table - 4: Performance of the broiler chickens fed the experimental diets.

Parameters	Guava leaf supplementation				Level of significance
	T_1 (0%)	T_2 (2.5 %)	T_3 (3.5 %)	T_4 (4.5 %)	
Initial body weight (g)	41.0± 6.4	39.9± 6.7	42.9± 6.7	38.5± 6.25	NS
Final body weight(g)	2164 ± 55.3	2064± 56.0	2095 ± 56.5	2026± 54.9	NS
Weight gain (g)	2113 ± 56.2	2013 ± 56.6	2044 ± 56.5	1975 ± 56.4	NS
Feed intake (gm/d)	3950 ± 57.8	3893 ± 57.2	4021 ± 57.3	3960 ± 57.4	NS
FCR	1.88 ± 0.07	1.95 ± 0.05	1.95 ± 0.04	2.00 ± 0.08	NS

- Values are expressed as mean ± standard error of means. NS: Statistically not significant ($P > 0.05$).
- Means represent three replicates, fifteen birds per replicate.
- FCR= Feed conversion ratio

Table - 5: Fat content and mortality percentage of the broiler chickens fed the experimental diets.

Parameters	Guava leaf supplementation				Level of significance
	T_1 (0%)	T_2 (2.5 %)	T_3 (3.5 %)	T_4 (4.5 %)	
Fat content (g)	78.0 ± 3.32^b	77.56 ± 1.87^b	72.45 ± 4.08^a	68.6 ± 3.45^a	*
Mortality (%)	3.56 ± 1.66^b	3.00 ± 2.21^b	2.87 ± 1.08^a	2.50 ± 1.90^a	*

- Values are expressed as mean ± standard error of means. a, b Means within row with different superscripts are statistically different (P <0.05).
- * Statistically significant (P<0.05).
- Means represents three replicates, fifteen birds per replication.

Table - 6: Bacterial organisms isolated from the navels of day-old-chicks.

Bacterial isolates	Broilers
E. coli	Present
Streptococcus sp	Present
Staphylococcus sp	Present

Table - 7: Antibacterial activity of guava aqueous leaf on organisms isolated from the navel of day-old chicks.

Bacterial isolates	Concentration of the Extract (mg/ml)	Zones of Inhibition (mm)
E. coli	400	25
	200	18
	100	16
Streptococcus sp	400	20
	200	16
	100	13
Staphylococcus sp	400	25
	200	20
	100	18

Table - 8: The minimum inhibition concentration of guava aqueous leaf extract against some of the isolated bacteria.

	Concentration of the Extract (mg/ml)				
Bacterial isolates	**50**	**25**	**15.5**	**6.25**	**3.13**
E. coli	-ve	-ve	-ve	+ve	+ve
Streptococcus sp	-ve	-ve	+ve	+ve	+ve
Staphylococcus sp	-ve	-ve	+ve	+ve	+ve

- +ve= with bacterial growth, -ve = without bacterial growth.

