EFFICACY OF GUAVA LEAVES (*Psidium guajava*) SUSPENSION ON GROWTH, ANTIBACTERIAL AND HEMATOLOGICAL PERFORMANCES IN *E. coli* INOCULATED JAPANESE QUAIL

A THESIS

BY

KRISHNA PRASAD MAHATO

REGISTRATION NO. 1605190

SESSION: 2016-2017

SEMESTER: JANUARY–JUNE, 2016

MASTER OF SCIENCE (MS)

IN

PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY & PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200, BANGLADESH

JUNE, 2018

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JUNE, 2018

Dedicated

To

My Beloved Parents

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ABSTRACT

The present study was designed to determine the growth, antibacterial and hematological effects of guava leaf (*Psidium guajava*) suspension in *E. coli* infected Japanese quail under the Department of Physiology and Pharmacology, HSTU, Dinajpur, Bangladesh, during September to October, 2017. A total of 40 quails of 10 days old were randomly assigned into four treatment groups named T_0, T_1, T_2, T_3 and each group contained 10 birds. GroupT_0 and T_1 were considered as negative and positive control, respectively. Group $T_2$and T_3 were treated with guava leaf (*Psidium guajava*) suspension and Doxycycline respectively. At 5 days interval, live body weights were recorded, bacterial loads in feces were counted and blood parameters were determined. Mortality was also observed in different treatment groups throughout the experiment. Body weights were significantly ($P<0.05$) increased in T_2 and T_3 group compared is $T_0$and $T_1$where as the bacterial load counts were significantly ($P<0.05$) decreased in T_2 and T_3compared is T_0 and T_1 groups. The present study was revealed that the mortality rate was significantly ($P<0.05$) higher in T_1 group. There were significant ($P<0.05$) variation of total leucocytes count neutrophil, lymphocyte and eosinophil among the different treatment groups. But, the eosinophil count was insignificantly ($P>0.05$) varied among the treatment groups on the day 22^{th} and 32^{th}.

Key words: - Guava leaves, quail, *E. coli*, antibiotics

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LIST OF ABBREVIATIONS

i.e. : That is

et al. : Associates with

g : Gram

etc. :et cetera

E. : Escherichia

P. : Psidium

%. : Percent

Spp. : Species

g. : Gram

HIV. : Human immunodeficiency virus

α. : Alpha

pH. : Pouvoir hydrogen

β. : Beta

Mg. : Milligram

Kg. : Kilogram

°C. : Degree centigrade

µg. : Microgram(s)

µL. : *Microlitre*

S. : *Salmonella*

DMSO. : Dimethyl sulphoxide

µg/mL. : Microgram per milliliter

MIC. : Minimum Inhibitory Concentration

MBC. : Minimum Bactericidal Concentration

@. *: At the rate of*

Ml. : Milliliter

EMB. : Eosin Methylene Blue

TVC. : Total Viable Count

CFU. :Colony forming units

TLC. : Total Leucocytes Count

DLC. : Different Leucocytes Count

Fig. : Figure

HSTU. : *Hajee Mohammad Danesh Science and Technology University*

L. *: Liter*

Ltd. *: Limited*

&. *: And*

<.	*: Less than*
>.	*: Greater than*
J.	*:* Journal
B	: Bacillus
GC	: Gas Chromatography
MS	: Mass Spectrometry
Linn	: Linnaeus
L	: Linnaeus
NB	: Nutrient Broth
temp	: Temperature
mm	: Millimeter
PBS	: Phosphate Buffer Solution

CHAPTER I
INTRODUCTION4

Quail is a small avian species belongs to the Pheasant family.In1595 first domesticated in Japan. Two species of quail found in India i.e. The Black breasted quail found in Jungle (*Coturnix Coromandelica*) other species is the Brown color Japanese Quail (*Coturnix Coturnix Japonica*) which is bred for meat or the one used for commercial Quail production. Quail is fast growing bird with a short generation gap. Quail were first introduced in India in 1974 from California (Mishra Priti and ShuklaSatish, 2014).Require minimum space for rearing. Require small capital. Quails are robust bird. Birds can sale at the early age of five weeks. It becomes mature at the age of six to seven weeks then start laying eggs. High rate of clutch up to 280.Quail meat is tasty other than chicken and has low fat content. It promotes body and brain development in young ones. Quail farming is a cheap enterprise compare to chicken farming. It is useful as choice of food. Quail is the important bird for scientific research. This species can be reared at interior places. It does not require the vaccination and medication. Quail litter has high fertilizer value and can be used for increasing yield of crops. Quail weighs up to 100 gm and lays 100 eggs a year, the Japanese quail weighs up to 250 gm and lays 250 eggs a year.

As per the nutritional criteria, the quail eggs are far better compare to that of chicken eggs. It has low cholesterol percentage. Quail meat and eggs are good for the pregnant women and infant feeding womens

(Mishra Priti and ShuklaSatish, 2014).Japanese quail (*Coturnixcoturnix japonica*) is a recent addition to the poultry farming in Bangladesh. Quail farming for egg and meat is quite popular in Japan, Hongkong, Korea, China, Singapore, India, Thailand, Malaysia, Indonesia, France, Italy, Germany, Britain and Russia. In Bangladesh it was introduced for the first time in 1990. There are about 131 species of wild quail found all over the world (Goetz, 1987). Only Bobwhite quail (*Colinusvirginianus*) and Japanese quail have been domesticated for commercial purposes. Japanese quail has several breeds and varieties of which Pharaoh (wild type), British Range, English White, Manchurian Golden, Tuxedo are most popular (Singh, *et al.,* 1982; Panda, *et al.,* 1987; Panda, 1990). Among these, Pharaoh is widely raised all over the world. It has two popular colour strains, wild colour and brown colour (Rahman, 1995).

In Bangladesh only these two are commercially available (Siddique and Mandal, 1996).The climate and natural condition of Bangladesh is also very suitable for quail rearing. Quail can be reared in this country throughout the year and shows a good performance in meat and egg production. It has a shorter life cycle and its production requires less capital and land. Quail may be a source of income in addition to chicken and ducks for its immense potentiality for meat and egg production (Paul and Sarker, 1992).

Escherichia coli is a part of the common microbial flora of the intestine of poultry and most isolates are nonpathogenic. About 10 to 15% of intestinal coliforms are pathogenic serotypes (Barnes and Gross, 1997). Pathogenic *E. coli* are also present in the poultry environment.

Escherichia coli causes a variety of lesions in poultry, including yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis (Gross, 1994). Colibacillosis is an economically important disease, which is prevalent throughout the world (Margie and Lawrence, 1999).

Several serotypes of *Escherichia coli* have been associated with disease conditions in poultry, the most common manifestation being colisepticemia (Sojka, 1965). *E. coli* 078:K80 is one of the serogroups most commonly isolated from affected birds (Hemsley*et al.,* 1967 and Sojka, 1965). *Escherechia coli* (*E.coli*) infection includes colibacillosis, Hjarre's disease coligranuloma, peritonitis, salpingitis, synovitis, omphalitis, air sac disease etc. Colibacillosis occurs as an acute fatal septicemia or subacute pericarditis and air sacculitis. It is a common systemic disease of economic importance in poultry and is seen worldwide. *E. coli* is a normal inhabitant of the intestinal tracts of animals and birds and is harmless as long as it is kept in check by other intestinal bacteria (Barnes *et al*., 2003) although most are nonpathogenic, a limited number produce extra-intestinal infections and its presence in drinking water is considered indicative of faecal contamination. *E. coli* persist for long period of time, particularly when dry rodents droppings often contain pathogenic coliforms.

Guava is a small tropical tree that grows up to 35 feet tall, it is widely grown for its fruit in tropics. It is a member of the Myrtaceae family, with about 133 genera and more than 3,800 species. The leaves and bark of *P.*

guajava tree have a long history of medicinal uses that are still employed today (Nwinyi *et al.*, 2008).

According to our socio-economic situation, the knowledge of our farmer is very little because most of them are not properly trained for poultry production, but unemployed young generation is coming in this business for short return of value and profit. Pharmaceutical companies take this advantage. They are convincing farmers for using antibiotics as a growth promoter or life savings for poultry. As a result, each and every poultry is a depot of antibiotics. When these poultry are consumed by human this antibiotic residue enters into human body and causing serious human health hazards with drug residues. Due to the prohibition of most of antimicrobial growth promoters (AGP), plant extracts have gained interest in animal feed strategies. Medicinal plants are cheap and renewable sources of pharmacologically active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile *et al.*, 1999).

Guava (*Psidium guajava* Linn.) commonly known for its food and nutritional values throughout the world. The medicinal properties of guava fruit, leaf and other parts of the plant are also well known in traditional system of medicine. Since, each part of guava tree possesses economic value, it is grown on commercial scale. Guava plant is considerable process has been achieved regarding the biological activity and medicinal application of guava and the fruit considered as poor man apple of tropics. The guava plant parts are used for the development of various industrial and pharmaceutical products (Priya, 2011).

Psidium guajava L. (guava), a fruit plant belonging to the family Myrtaceae, is found all over the world. Guava leaves, roots, and fruits have been used for the prevention and treatment of diarrhea (Lutterodt, 1989; Alnieida *et al*., 1995). In several studies, guava showed significant antibacterial activity against common food borne diarrhea-causing bacteria such as *Staphylococcus spp., Shigella spp., Salmonella spp., Bacillus spp., E. coli, Clostridium spp.,* and food spoilage bacteria such as *Pseudomonas spp* (Alnieida *et al*., 1995; Jaiarj *et al*., 1999; Farhana *et al.,* 2017).

Guava leaves have long been recognized for their antimicrobial activity (Bansode and Chavan, 2014). Guava leaves have several chemical constituents such as comarins, essential oils, flavonoids, triterpenes and ellagitannins which are known to have antimicrobial properties (Sapkota *et al.,* 2012).

In view of above facts, the present study was undertaken with a view to fulfilling the following objectives:

1. To know the effect of Guava leaf suspension on body weight, blood parameters in quail infected with *E. coli*.
2. To know the effect of Guava leaves suspension against *E. coli.*
3. To differentiate the antibacterial effect of Guava leaf from synthetic drug.

CHAPTER II
REVIEW OF LITERATURE

2.1 Quail

Quail is a small avian species belongs to the Pheasant family. In 1595first domesticated in Japan. Two species of quail found in India i.e. The Black breasted quail found in Jungle (*Coturnix Coromandelica*) other species is the Brown color Japanese Quail (*Coturnix coturnix Japonica*) which is bred for meat or the one used for commercial Quail production. Quail is fast growing bird with a short generation gap. Quail were first introduced in India in 1974 from California (Mishra Priti and Shukla Satish, 2014).

The quail also known as Bater in hindi terminology. It is a small medium size game bird related with pheasant family. In India two species occurs namely Black breasted quail found in jungle (*Coturnix coromandelica*) and other one Brown color Japanese quail (*Coturnix coturnix Japonica*), which is bred for meat or the one used for commercial quail production. A broiler (meat purpose) quail can be sold at 5 weeks. Quails start laying eggs at about 6 weeks to continue to give high egg production up to 24 weeks of age. Adult Japanese quail weigh up to 250 g and lays 250 eggs a year. The meat is used as ready to cook meat, pickled meat & tandoori quail. From the quails egg can make different recipes like Boiled egg and egg pickles. The egg size is about 10 g. It requires smaller house for rearing. About 10 quails require space is equal to require space for one chicken (Mishra Priti and ShuklaSatish, 2014).

Japanese quail (*Coturnix coturnix japonica*) is a recent addition to the poultry farming in Bangladesh. Quail farming for egg and meat is quite popular in Japan, Hongkong, Korea, China, Singapore, India, Thailand, Malaysia, Indonesia, France, Italy, Germany, Britain and Russia. In Bangladesh it was introduced for the first time in 1990. There are about 131 species of wild quail found all over the world (Goetz, 1987; Siddique and Mandal, 1996)

The climate and natural condition of Bangladesh is also very suitable for quail rearing. Quail can be reared in this country throughout the year and shows a good performance in meat and egg production. It has a shorter life cycle and its production requires less capital and land. Quail may be a source of income in addition to chicken and ducks for its immense potentiality for meat and egg production (Paul and Sarker, 1992; Siddique and Mandal, 1996).

A large number of small-scale commercial quail farms or 'QUAILARY have been established in Bangladesh in the recent years. The present study is based on primary data collected from 76 quail farms in Dhaka metropolitan city. The findings of the study clearly indicate that the large layer quail farms are more profit earners than the small layer farms. The results also show that chicks or pullet production is highly profitable, compared to egg production. Cobb-Douglas production function analysis suggests that most of the selected variables had significant impact on the quail farmers' return. The study identified a number of problems of raising quail such as high prices of feed, inadequate institutional credit, lack of veterinary services and medicine, lack of training on quail

husbandry and inadequate product marketing facilities (Siddique and Mandal, 1996).

2.2 Nutritional benefit of quail egg

Cross-sectional study of quail eggs was conducted to evaluate the nutritional compositions of carbohydrate, fat, protein, calories, vitamin, minerals and sex hormones. The results showed that average of each whole quail egg weight was 10.67 g. Their contents of ash, carbohydrate, fat, protein and moisture were 1.06, 4.01, 9.89, 12.7 and 72.25 g 100g-1, respectively. Total energy in calories obtained was 156.50 kcal 100g-1 whole egg. The most essential amino acid found in egg whites, was leucine and the most non-essential amino acid was aspartic acid. Egg yolks contained the highest essential fatty acid content of linoleic acid and the highest non-essential fatty acid content of oleic acid. In addition, there was high content of vitamin E in egg yolks and sex hormone progesterone in both of egg yolks and whites. The most essential and trace minerals of whole eggs were nitrogen and iron. Iron was high content in egg whites meanwhile nitrogen and zinc were found high in egg yolks. This study indicated that quail eggs contained high nutritional contents of amino acids, fatty acids, vitamin E, sex hormone and minerals of nitrogen, iron and zinc. Quail eggs are the good source of nutrients for human health. (Tunsaringkarn *et al.*, 2013).

2.3 Guava

2.3.1 Common names

Common guava, yellow guava, apple guava Tagalog: Bayabas, kalimbahin, tayabas, guayabas. Bisayan: Bayabasllokano: Bayabas, guayabas.

2.3.2 Description

Guava is a tropical and semitropical plant. It is well known in the islands for its edible fruit. It is common in the backyards. The branches are crooked, bringing opposite leaves. The flowers are white, incurved petals, 2 or 3 in the leaf axils. The fruit is small, 3 to 6 cm long, pear-shaped, reddish-yellow when ripe (Ticzon, 1997).

The guava tree (*Psidium guajava* L.) belongs to the family Myrtaceae. It is considered to be a native Mexican plant, but has spread throughout South America, Europe, Africa, and Asia (Shah *et al.,* 2011). Because it adapts to different climate conditions, it grows in all tropical and subtropical regions of the world; however, it is widespread in Brazil (Gutierrez *et al*., 2008).

2.3.3 Active ingredients

Numerous tannins and other phenolic compounds have been identified from *P. guajava*, of which amritoside is of particular importance. Amritoside is a glycoside (gentiobioside) of ellic acid. Another biologically interesting compound in the plant is guiajaverin, a glycoside (arabinopyroside) of quercetin. The leaves also contain essential oils and triterpenoids (Ayensu, 1978).

The active compounds found in guava leaves were alkaloid, saponin, tannin, phenol, flavonoid, triterpenoid, and steroid. (Romasi *et al*., 2006).

2.3.4 Pharmacological effects:

Ellagic acid is a known intestinal astringent and haemostatic which explains the therapeutic value of the plant against diarrhoea and dysentery. The tannins are generally of value because of their vasoconstricting effects and their ability to form a protective layer on the skin and mucosas. These effects, together with proven antibacterial and antifungal activity, result in effective treatment of both internal and external infections. Quercetin (and its glycosides) undoubtedly also contributes to the efficacy of the medicine, because it is a known antioxidant with anticarcinogenic, anti-HIV and antibiotic effects. Hypoglycaemic effects have been documented (Ayensu, 1978).

2.3.5 Chemical composition

The main constituents of the essential oil of *Psidium guajava* are limonene (2.24.4%), trans-caryophyllene (18.1-17.1%), α-humulene (26.320.4%), aromadendrene (7.6-12.2%), α-selinene (7.3-11.3%), caryophyllene oxide (3.7-3.3%), humulene epoxide II (4.1-1.9%), and selin-11-en-4α-ol (7.2-11.1%). Chemical classes that underwent major changes with respect to collection time were monoterpenes, sesquiterpenes, and sesquiterpenoids (2.2-4.4%, 63.8-61.7%, and 15.9-13.2%, respectively) (Silva *et al.,* 2016).

The chemical composition of the biomass (branches and leaves) *Psidium guajava* L. generated in the pruning practices as follows: pH (4.985.88), soda solubility (39.01-70.49 %), ash (1.87-8.20 %); potassium and calcium were the major inorganic elements in ash. No heavy metals were detected in the studied samples; total solubility (15.21-46.60 %), Runkel lignin (17.77-35.26%), holocellulose (26.56 -69.49 %), α-cellulose (15.53-

35.36 %), hemicelluloses (11.0234.12 %), tannins in aqueous extracts (3.81-9.06 %), and tannins in ethanolic extracts (3.42-15.24 %) (Camarena-Tello *et al*., 2015).

The chemical composition of the essential oils from leaves and stems of *Psidium guajava,* grown in Tunisia were determined by gas chromatography coupled to mass spectrometry GC–MS. Twenty-one compounds were determined. The major compounds identified in the oil of the stems were α-humulene (10.93%), Germacrene D (16.79%) and Valerenol (10.62%), whereas leaf oil was dominated by Veridiflorol (36.4%) and Trans-caryophyllene (5.9%). The yield of the oil was 0.66% (v/w) (Khadhri*et al*., 2014).

Flavonoid content of *Psidiumguajava* leaves has been observed to have antibacterial activities (Rattanachaikunsopon and Phumkhachorn, 2010).

Arya1 *et al.,* (2012) carried out a study to investigate the phytochemical profile of leaves of *Psidium guajava* L. The leaves powder was successively extracted with petroleum ether, chloroform, ethanol, water, hydroalcoholic. Phytochemical analysis shows the presence of flavonoids, tannins triterpenoids, saponins, sterols, alkaloids and carbohydrates.

The main constituents of guava leaves are phenolic compounds, isoflavonoids, gallic acid, catechin, epicathechin, rutin, naringenin, kaempferol. The pulp is rich in ascorbic acid, carotenoids (lycopene, β-carotene and β-cryptoxanthin). The seeds, skin and barks possess glycosids, carotenoids and phenolic compounds (Barbalho *et al*., 2012).

The main constituents of guava are vitamins, tanins, phenolic compounds, flavonoids, essential oils, sesquiterpenealcohols and

triterpenoid acids. These and other compounds are related to many health effects of guava (Haida *et al*., 2011).

Chemical composition of various parts of Guava (*Psidiumguajava* Linn.)

Parts	Constituents	Reference
Fruit	Vitamin C, vitamin A, iron, calcium, Manganese, phosphoric, oxalic and malic acids, saponin combined with oleanolic acid. Morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside, flavonoids, guaijavarin, Quercetin. Essential oil contains hexanal , -2-hexenal , 2,4-hexadienal, 3-hexenal, 2-hexenal, 3hexenyl acetate and phenol, while β-caryophyllene, nerolidol , 3-phenylpropyl acetate, caryophyllene oxide, pentane-2-thiol, 3-penten-2-ol and 2-butenyl acetate, 3-hydroxy-2-butano3-methyl-1-butanol, 2,3butanediol, 3-methylbutanoic acid, (Z)-3-hexen-1-ol, 6methyl-5-hepten-2-one, limonene, octanol, ethyl octanoate (pink guava fruit).	Hernandez *et al*., 1971; Iwu, 1993; Burkill, 1997; Nadkarni and Nadkarni, 1999; Bassols, Demole, 1994; Paniandy*et al*., 2000.
Leaves	α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene, mallic acids, nerolidiol, β-sitosterol, ursolic, crategolic, and guayavolic acids, cineol, quercetin, 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside (Essential oil), resin,	Zakaria*et al.,* 1994, Iwu 1993, Nadkarni and Nadkarni, 1999; Oliver Bever, 1986; Begum *et al.,* 2002; Wyk*et al*., 1997, Joseph *et al.,* 2010

	tannin, eugenol, caryophyllene (1a α-, 4a α-, 7 α-, 7a β-, 7b α)]-decahydro-1H-cycloprop[e] azulene, Guajavolide (2 α-,3 β-,6 β-,23-tetrahydroxyurs-12-en-28,20 β-olide; 1) and guavenoic acid (2 α-,3 β-,6 β-,23-tetrahydroxyurs12,20(30)-dien-28-oic acid, triterpeneoleanolic acid, triterpenoids, flavinone-2 2'-ene, prenol, dihydrobenzophenanthridine and cryptonine.	
Seed	Proteins, starch, oils, phenolic, flavonoid compounds, flavonol glycoside, quercetin-3-O-β-D-(2"-Ogalloyglucoside)-4'-O-vinylpropionate	Michel *et al.,* 2002; Burkill, 1997
Bark	polyphenols, resin and crystals of calcium oxalate	Burkill, 1997; Nadkarni and Nadkarni, 1999
Root	Tannin, leukocyanidins, sterols, gallic acid, carbohydrates, salts, tannic acid.	Iwu 1993, Quisumbing, 1978

2.3.6 Medicinal Uses

The leaves of the guava tree in decoction are recommended for gastrointeritis, uterine hemorrhage, chronic diarrhea, swollen legs, etc. The young leaves and shoots are used for dysentery, inflammation of the kidney, and diarrhea. The same decoction is good as a wash for ulcers, vaginal and uterine problems, and where an astringent remedy is needed. It heals wounds and cuts. It has been used for spasms, fevers, worms, kidney problems, epilepsy, diabetes and even for cerebral affections.

P. guajava is a well known traditional medicinal plant used in some indigenous systems throughout the world. All parts of this tree, including roots, bark, leaves, seeds, and the fruits have been used for treatment

gastrointestinal problems. Leaves, pulp and seeds are used as an antispasdomic, antiinflammatory, and anti-diarrheic, to treat respiratory and gastrointestinal disorders, in the treatment of hypertension, obesity and in the control of diabetes mellitus (Barbalho*et al*., 2012). It also possesses anticancer properties (Ryu *et al.,* 2012). The seeds of *P. guajava* are also used because of their antimicrobial, gastrointestinal, anti-allergic, and anti-carcinogenic activities (Pelegrini *et al.,* 2008; Metwally *et al.,* 2010; Huang *et al.,* 2011; Bontempo *et al.,* 2012).

All parts of the guava plant have been used for different purposes: hepatoprotection, antioxidant, anti-inflammatory, antispasmodic, anti-cancer, antimicrobial, anti-hyperglycemic, analgesic, endothelial progenitor cells, anti-stomachache and anti-diarrhea. *P. guajava* has many effects on health and that it should be researched more extensively in clinical trials. Furthermore leaves, seeds and peel are treated as wastes by the food processing industry and are discarded, so their use may reduce the disposal of these parts of guava as pollutants (Barbalho *et al.,* 2012).

Essential oils distilled from aromatic and medicinal plants have been used both cosmetically and therapeutically. Nowadays, *Psidium guajava* L. plant parts are commonly used as medicinal plant. Although, there is considerable anecdotal information about the biological activity of guava essential oils much of this has not been substantiated by scientific evidence. Among the claims made for *Psidium guajava* (L.) essential oil is that have antimicrobial, antinociceptive, repellent, insecticidal, anticancer and anti-inflammatory effects. In this study we detail the

current state of knowledge about the effect of guava essential oils and phytochemicals (JosephandPriya, 2011).

The bark is used as an astringent in the treatment of ulcers wounds, diarrhea, dysentery, skin ailments, vaginal hemorrhage wounds, fever, dehydration, and respiratory disturbances; the root is used as a decoction to treat diarrhea, coughs, stomach ache, indigestion, toothaches, and constipation; the whole plant is in general used in the form of decoction, infusion and paste as skin tonic (Gutiérrez *et al.,* 2008).

Extracts of roots, bark, and leaves Guava or *Psidium guajava* are used to treat gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions (Morton 1987).

Guava is commonly used as a medicine against gastroenteritis and child diarrhea by those who cannot afford or do not have access to antibiotics (Farhana *et al.,* 2017).

Guava leaf tea of *Psidium guajava* Linnaeus is commonly used as a medicine against gastroenteritis and child diarrhea by those who cannot afford or do not have access to antibiotics (Gonçalves *et al.*, 2008).

Guava (*Psidium guajava* L.) leaf extract was used as a functional ingredient for immunostimulant (NoerLaily *et al.,* 2015).

Guava leaf extract has analgesic, anti-inflammatory, antimicrobial, hepatoprotective and antioxidant activities. These effects are probably due to the presence of phenolic compounds (Ryu *et al.*, 2012; Roy *et al.*, 2006).

Birdi *et al*. (2010) and Birdi *et al*. (2011), related that *P. guajava* leaves have a broad spectrum of antimicrobial action (as antigiardial and antirotaviral activity) that could be effective in controlling diarrhea due to a wide range of pathogens. The antimicrobial activity may be due to the presence of flavonoids extracted from guava leaves (Rattanachaikunsopon and Phumkhachorn , 2010; Dhiman *et al*., 2011).

Jiménez-Escrig*et al*.(2001), Wang *et al.*(2007) and Haida *et al.*(2011), reported the presence of higher amounts of phenolic compounds with antioxidant activity in the leaves of white (*Psidium guajava* var. *pyrifera* L.) and red guava (*Psidium guajava* var. *pomifera* L.) when compared with other vegetable species. Wu *et al.* (2008) and Melo *et al*. (2011), found gallic acid, catechins, epicatechins, rutin, naringenin and kaempferol in the leaves.

2.3.7 Antibacterial activity

A study was designed to examine the efficacy of ethyl acetate fraction of aqueous extracted *Psidium guajava* leaves on chicks expermentally-infected with diarrheagenic strain of *Escherichia coli* O78. A total of 60 ISA brown male chicks were randomly divided into 6 Groups of ten chicks each in separate cages. Group A was not infected and not treated. Groups B, C and D were infected and treated with extracts at a dose of 25, 50 and 100 mg/kg respectively for 10 days. Group E was infected and treated with oxytetracycline while Group F was infected, but left untreated. Chicks from all groups were closely monitored for clinical signs, body weight change and fecal bacteria shedding load during the course of the experiment. Diarrhea, vents pasted with feces, drop in feed

intake accompanied by slow weight gain and decreased activity was observed in infected untreated groups. Groups treated with graded doses of the extract experienced a dose-dependent decreased in severity of the clinical signs shown compared to the infected untreated group. Bacterial shedding load was found to be lower in groups treated with the extract and oxytetracycline than those without intervention. Ethyl acetate soluble fraction of leaf extract of *Psidium guajava* effectively controlled diarrhea and decreased the severity of other clinical signs caused by experimental *E. coli* infections in chicks (Geidam*et al.*, 2007).

Psidium guajava Linn. leaf extract containing phenolic compounds are known for antimicrobial activity (Katewaraphorn and Aldred, 2016).

A study was conducted to clarify the possible effects of antimicrobial activities of guava extracts, determined against five food-borne pathogens: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (BTCC 19), *Shigella sonnei* (BTCC) and *Salmonella typhi* (BTCC 197) using disc diffusion method at four different concentrations: 10%, 50%, 75% and 100%. Its antibacterial activity was also determined at three different temperatures: 50°C, 75°C and 100°C. The test organisms differed in their reaction to these different extracts, but as a whole inhibition of bacterial growth increased with the increased concentration. All the samples showed antibacterial activity after heat treatment at 50°C, 75°C and 100°C suggesting that the temperature does not affect the activity. Guava extracts showed higher antibacterial activity against gram positive bacteria compared to gram negative

bacteria. None of the extracts (10%) showed antibacterial activity against these pathogens (Farhana *et al*., 2017).

The antimicrobial effect of essential oils and methanol, hexane, ethyl acetate extracts from guava leaves was studied against diarrhea-causing bacteria: *Staphylococcus aureus*, *Salmonella* spp. and *Escherichia coli*. Strains that were screened included isolates from seabob shrimp, Xiphopenaeuskroyeri (Heller) and laboratory-type strains. Of the bacteria tested, *Staphylococcus aureus* strains were most inhibited by the extracts. The methanol extract showed greatest bacterial inhibition. No statistically significant differences were observed between the tested extract concentrations and their effect. The essential oil extract showed inhibitory activity against *S. aureus* and *Salmonella spp*. Guava leaf extracts and essential oil are very active against *S. aureus*, thus making up important potential sources of new antimicrobial compounds (Gonçalves *et al*., 2008).

Biswas *et al.,* (2013), determined the antimicrobial potential of *guava(Psidium guajava)* leaf extracts against two gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) and two gram-positive bacteria (*Staphylococcus aureus and Bacillus cereus*) which are some of food borne and spoilage bacteria. The guava leaves were extracted in four different solvents of increasing polarities (hexane, methanol, ethanol, and water). The efficacy of these extracts was tested against those bacteria through a well-diffusion method employing 50□L leaf-extract solution per well. According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed

inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3mm, and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0mm against *B. cereus* and *S. aureus* respectively.

Gurnani *et al.,*(2016), assessed the effect of 5%, 10%, 15% and 20% concentration of ethanol, DMSO (Dimethyl sulphoxide), and water extracts of guava leaves against *Lactobacillus acidophilus*. Extracts of guava leaves with ethanol, water & DMSO were prepared by using Soxhlet extractor. Four concentrations 5%, 10%, 15% and 20% weight/volume of ethanol, water & DMSO extracts were prepared. Agar well diffusion method was employed to test the antibacterial efficacy. 8 plates each were prepared for the three extracts. Chlorhexidine (0.2) &distilled water were used as positive & negative control. Results: Only two extracts i.e. ethanol & water of *P. guajava* & 0.2% chlorhexidine showed activity against both *L. acidophilus*. Maximum zone of inhibition was observed with CHX. Mean zone of inhibition produced by 0.2% chlorhexidine was 22.25mm & by 20%, 15%, 10%and 5% ethanolic extract was 21.34mm, 17.56mm, 16.14mm and 15.34mm respectively, and least activity was shown by water extract & no zone of inhibition was observed with DMSO extract. 20% ethanolic extract of guava was found as efficacious as 0.2% chlorhexidine.

The synergistic inhibition effect of the antibiotics Tetracycline with local guava, noni, carambola and Kariyat extracts on in vitro growth of diarrheal pathogens; *Escherichia coli* and *Salmonella sp.,* clinically

isolated from Yingo Hospital, Yingo District, Narathiwat Province, Southern Thailand was investigated using broth dilution technique. The solvnts of herbal extraction included acetone, hexane, methanol, ethanol, and water. Results showed different degree of inhibition against *E. coli* and *Salmonella sp*. with the Minimum Inhibitory Concentration (MIC) values of 6.25 and 9.37µg/mL, and the Minimum Bactericidal Concentration (MBC) values of 25 and 37.5µg/mL, respectively. As for herbal extracts, growth inhibition of *E. coli* was observed with the MIC lowest value of 3.12 µg/mL (acetone-guava, hexane-guava, acetone–Kariyat extracts), and the lowest MBC of 25 µg/mL (acetone–kariyat extract). For *Salmonella sp.*, it was found that the lowest MIC values was of 6.25 µg/mL (acetone-guava, methanol-guava, acetone-Kariyat, hexane-Kariyat, methanol-Kariyat, ethanol-Kariyat, methanol-carambola, ethanolcarambola, methanol-noni extracts), and the lowest MBC value was 50 µg/mL (acetone-noni, acetone-carambola, hexane-noni, hexane-carambola, hexane–guava, ethanol–noni, ethanol–guava extracts). Synergistic inhibiting effect of the tested herbal extracts with Tetracycline showed efficient results with the lowest MIC and MBC values for *E. coli* and *Salmonella sp* were of 0.78 µg/mL and 0.78 µg/mL, respectively. Synergistic effect of herbal extracts with Tetracycline was thus clearly shown, and therefore, of potential in clinical application after thorough and further detailed in vitro and in vivo investigation (Dalee *et al.*, 2016).

CHAPTER III
METERIALS AND METHODS

3.1 Study area and study period

The present study was conducted during september to October, 2017 in the research unit under the Department of Physiology & Pharmacology at Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

3.2 Study birds

A total number of 40 quails were collected from Bahadur Bazar, Dinajpur, Bangladesh.

3.3 Collection and management of quails

At 10 days of age, 40 Japanese quails were used for study. They were collected from Bhadur Bazar, Dinajpur, Bangladesh and fed with quail commercial (Power Feed Ltd., Gagipur, Bangladesh) and water ad libitum. The quails were allowed to acclimatize in their new environment for 6 days before the commencement of the experiment. After collection, glucose and vitamin C were supplied with drinking water for three days. They were divided into 4 Groups (Groups To, T_1, T_2, & T_3) and were kept in separate quail cages. The body weights of assigned quails were taken with digital weight balance and the data were recorded. Also feces were collected for the examination of bacteria colony count.

3.4 Experimental designs

The quails were randomly divided into 4 equal Groups (T_0, T_1, T_2, T_3) in which each group consisted of 10 birds for assessing the efficacy of

guava leaves extracted juice and antibiotic as mortality, hematological parameter, postmortem, bacterial load and growth performance of quail.

Group T_0 was kept on –ve control (no supply *E. coli* & Guava leaves).

Group T_1 was +ve control (supply *E. coli* bacteria but no Guava leaves).

Group T_2 was supplied *E. coli*& were treated with Guava leaves juice @ 1.5 ml per 100 ml drinking water for 10 days.

Group T_3 was supplied *E. coli*& were treated with Antibiotic (Doxycycline) @ 1 g per 2L drinking water for 10 days.

LAYOUT OF THE EXPERIMENT

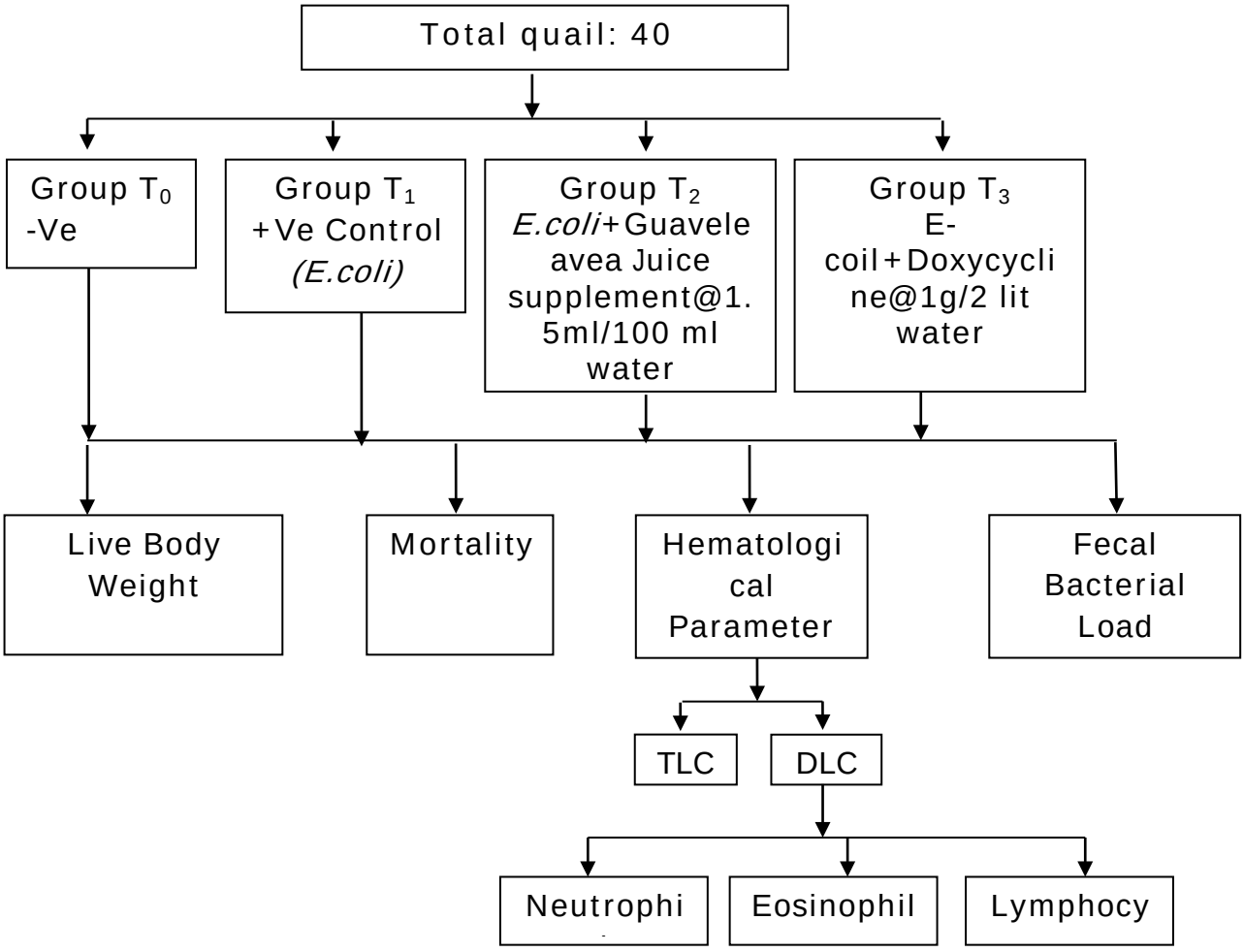


Fig 1: Layout of the experimental design (each group consisting of ten quails)

3.5 Test organisms collection and preparation

The test organism (*E. coli*) was collected from the laboratory under the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

Nutrient broth (NB) was used to grow the organisms from the collected samples before feeding the quails orally (Cheesebrough, 1985).

Fig 2: Japanese quail in experimental shed

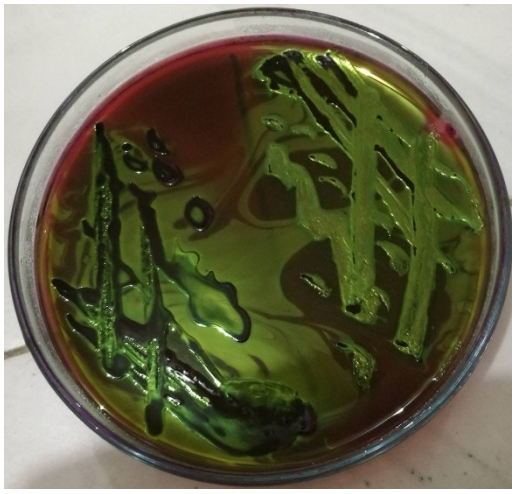


Fig 3: Test organism metallic sheen product by *E-coil* on EMB agar

3.5.1 Preparation of nutrient broth

3.5.1.1 Requirements

Bacto-nutrient broth (Difco)..... 13.0 g

Distilled water..... 1000 ml

3.5.1.2 Procedure

13.0 grams of Bacto-nutrient broth (Difco) was dissolved in 1000 ml of cold distilled water and heated up to boiling to dissolve it completely. The solution was then distributed in tubes, stoppered with cotton plugs and sterilized in the autoclave machine at l21°C and 15 pounds pressure per square inch for 15 minutes. The sterility of the medium was judged by incubating overnight at 37°C and used for cultural characterization (Carter, 1979).

3.5.2 Inoculation of organism

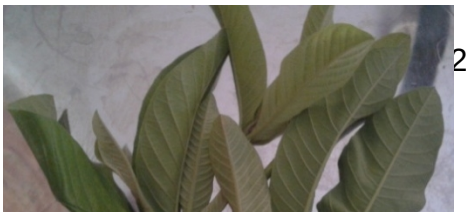
The organism (*E. coli*) was inoculated into nutrient broth media by metallic loop and was incubated for overnight in incubator at 37^0C temp.

3.5.3 Feeding of organism

The nutrient broth culture was shaked properly and the quails of groups T_1, T_2& T_3 were inoculated orally with 2-3 drops of the inoculums at 6^{th} day after reasting period.

3.6 Collection and processing of plant materials

Guava leaves were collected from the HSTU, Dinajpur, Bangladesh. The collected young guava leaves were washed in the tap water and the



fleshy parts were mashed with the help of pestle and morter. The guava leaf juice was extracted from mashed leaf.

Fig 4: Guava LeaveFig 5: Mashed of

3.7 Feeding of experimental diet

The guava juice was supplied to the quails of T_2 group with drinking water @ 1.5 ml per 100 ml drinking water for 10 days.

Doxycyline antibiotic was supplied to the quails of T_3 group with drinking water@ 1g per 2L drinking water for 10 days.

3.8 Recording body weight

Body weight of each bird was recorded at five days interval with the help of digital balance.

Fig 6: Measurement of body weight

3.9 Estimation of bacterial loads

A number of total four (4) feces samples were collected directly from different groups T_0, T_1, T_2& T_3. The samples were brought to the bacteriology laboratory, Department of Microbiology, HSTU, Dinajpur, Bangladesh and processed for the bacteriological colony examination.

3.9.1 Preparation of culture media

3.9.1.1 Eosin Methylene Blue (EMB) agar medium

Eosin methylene blue (EMB) agar medium was used to observe the growth of *Escherichia coli* (Cheesebrough, 1985).

3.9.1.2 Requirements

EMB agar base (Hi-media, India)..... 36.0 g

Distilled water ..... 1000 ml

3.9.1.3 Procedure

36.0 grams of EMB agar base (Hi-media, India) was added to 1000 ml of distilled water in a conical flask and heated until boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in to sterile glass petridishes. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used or stored at 4°C in refrigerator for future use (Carter, 1979).

3.9.2 Serial dilution of sample

The number of total four feces samples were weighted 1 g individually on digital weighting balance and diluted into 100 ml PBS solution as serial 10 fold dilution.

Fig 7: Prepared Serial dilution

Serial 10 fold dilutions of each of the feces samples in a series of dilution tubes were prepared. At first for each of the feces samples 10 sterile test tubes were placed on a test tube holder rack containing 9 ml of 2% buffered peptone water.

1 ml feces sample was mixed with 9 ml of Phosphate buffer solution in the 1st test tube in order to make 10-1 dilution. Then 1ml solution from 1st test tube mixed with 2ndt test tube, then from 2nd test tube to 3rd test tube and finally 9th to 10th test tube and 1ml discard from 10th test tube by the help of pipette and in every steps mixing was done properly.

3.9.3 Enumeration of Total Viable Count (TVC)

For the determination of total viable bacterial count, 1 ml of each ten-fold dilution was transferred and spread on duplicate plate count agar using a fresh pipette for each dilution. The diluted samples were spread as

quickly as possible on the surface of the plate with a cotton bud. One cotton bud was used for each plate. The plates were then kept in an incubator at 37 for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted by the digital colony counter machine. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to Icrate MSF 1998. The results of the total bacterial count were expressed as the number of organism or colony forming units per ml (CFU/ml) of feces sample.

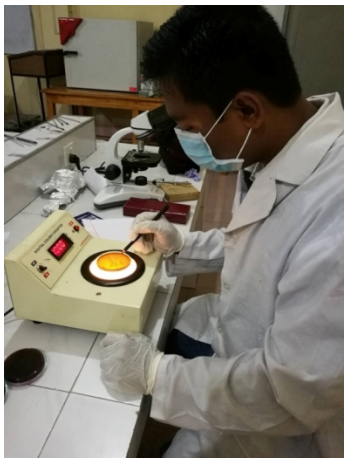


Fig 8: Counts of Bacterial colony

3.10 Haematological parameters

0.5 ml blood from each group was collected from wing vein with the help of syringe (1 ml) and needle. The collected blood was sent to the Taufiq Agro Lab, Rangpur, Bangladesh for the estimation of different blood parameters such as TLC (Total Leucocytes Count) and DLC (Different Leucocytes Count). The blood parameters were determined by semi automatic haematological analyzer machine (cureinc. U.S.A.).

Fig 9: Blood Collection of Quail from wine vein

3. 11 Recording mortality percentages

The quail cages were observed everyday and the number of death bird was recorded on the recording note book. The mortality percentage was calculated by the following formula:

$$\text{Mortality (\%)} = \frac{\text{No. of death bird}}{\text{Total no. of bird}} \times 100$$

3.12 Statistical analysis

Data were analyzed by analysis of variance using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986) using Completely Randomized Design (CRD). Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Performed with the help of SPSS 20 software to find out the difference among the treatments.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Effect of treatment with guava leaf suspension and Doxycycline on the body weight of quails infected with *E. coli*

Table 1 shows the various treatments on the body weight of quail. *E. coli* infection affected body weight gain of infected quail. There was significantly ($P<0.05$) increased in body weight in T_2 (guava leaf extract) and T_3 group (Doxycycline) than T_0 (negative control) and T_1 (positive control).At the age of 32 days the highest body weight was found in T_3 group (98.30±1.66) followed by T_2 (96.50±1.95), T_0 (89.20±1.34) and T_1 (80.80±3.70).The present results are more or less similar to the study of Geidam *et al., (*2015), who reported that body weight was found higher in guava extractand oxytetracycline than group infected with *E. coli*. El-Sayed *et al.,*(2013), also reported that guava leaves Roda significant improved effect on body weight and weight gain and FRC in broiler. On the other hand, some researchers (Rattanaphol and Rattanaphol, 2009) and (Wedy, 2012) declared that use of 0.04% or 0.06% of guava leaves extract in poultry ration didn`t have significant effect on body weight and weight gain.

Table1: Average body weight (Mean±SEM) of quail infected with *E. coli* and treated with guava leaf suspension and Doxycycline

Age (Days)	T_0	T_1	T_2	T_3	Level of significance
17	30.20±1.85	30.30±1.89	29.50±1.57	29.80±1.55	NS

	a	a	a	a	
22	50.00±1.89 a	44.10±2.62 a	49.90±1.83 a	49.80±1.68 a	NS
27	70.56±1.61 b	63.40±2.39 a	77.30±2.37 c	79.00±2.45 c	*
32	89.20±1.34 b	80.80±3.70 a	96.50±1.95 c	98.30±1.66 c	*

Note: Values are expressed as mean± standard error of means. Means between column are statistically significant (P<0.05) *= Significant at 5% level of significance. NS means statistically not significant.

4.2 Effect of treatment with guava leaf suspension and Doxycyclineon the faecal bacterial load count of quails infected with *E. coli*

The results of average bacterial load count in the feces of quail are shown in table 2.The present results indicate that bacterial shedding load was significantly (P<0.05) increased in T_0 and T_1 but significantly (P<0.05) decrease in T_2 and T_3 group in relation to age of quail. The bacterial load count in T_0 group was 88.00±1.03, 88.00±1.03, 92.00±1.03 and 96.00±1.03at day 17, 22, 27 and 32 respectively. In T_1 group the bacterial load was184.00±1.03, 188.00±1.03, 200.00±1.03 and196.00±1.03 at day 17, 22, 27 and 32 respectively. In T_2 group the bacterial load was 184.00±1.03, 196.00±1.03, 164.00±1.11 and 96.00±0.79 at day 17, 22, 27 and 32respectively. In T_3 group the bacterial load was 184.00±1.03, 156.00±1.03, 76.00±1.03 and 72.00±1.03 at day 17, 22, 27 and 32 respectively. The present results are in the line of the observation of Geidam *et al.*, (2015), who observed that bacterial shedding load was significantly lower in groups treated with guava leaf extract and oxytetracycline than those without intervention in

chickens. Vieira *et al.,* 2001 have also reported antibacterial effect of guava leaves extract and found that they inhibited growth of bacteria. Similar results are also observed by Mohammad *et al.,* 2012. Who reported that significant antibacterial activity against *S. aureus* and *E. coli.*Gitika and Kumar (2016). Also reported that guava leaves extract had antibacterial effect against *E. coli.*

Table 2: Mean bacterial colony count (Mean±SEM) of quail infected with *E. coli* and treated with guava leaf suspension and Doxycycline

Group	Age (Days)				
	17	22	27	32	Level of significance
T_0	88.00 ± 1.03^{a}	88.00 ± 1.03^{a}	92.00 ± 1.03^{b}	96.00 ± 1.03^{c}	*
T_1	184.00 ± 1.03^{a}	188.00 ± 1.03^{b}	200.00 ± 1.03^{d}	196.00 ± 1.03^{c}	*
T_2	184.00 ± 1.03^{c}	196.00 ± 1.03^{d}	164.00 ± 1.11^{b}	96.00 ± 0.79^{a}	*
T_3	184.00 ± 1.03^{d}	156.00 ± 1.03^{c}	76.00 ± 1.03^{b}	72.00 ± 1.03^{a}	*

Note: Values are expressed as mean± standard error of means. Means between column are statistically significant (P<0.05) *= Significant at 5% level of significance.

4.3 Effect of treatment with guava leaf suspension and Doxycycline on mortality (%) of quails infected with *E. coli*

The mortality (%) among the different treatment groups is shown in table 3. The present study revealed that mortality (%) was significantly (P<0.05) higher in T_1 (40%) group than others. But in guava leaf extracted group (T_2), there was found mortality (%) zero (0%). In T_3 group 1 (10%) quail was died before treatment. Birdi *et al*., (2010) and Birdi *et al*., (2011), reported that guava leaves have a broad spectrum of antimicrobial action. Biswas *et al*., (2013), also determined antibacterial potential of guava leaf extract against *E. coli* and *Salmonella enteritidis*.

Table 3: Effect of treatment with guava leaf suspension and Doxycycline on mortality (%) of quails infected with *E. coli*

Parameter	T_0	T_1	T_2	T_3	χ^2	Level of significance
Mortality (%)	1 (10%)	4 (40%)	0 (0%)	0 (0%)	9.83	*

Note: Values are expressed as mean± standard error of means. Means between column are statistically significant (P<0.05) *= Significant at 5% level of significance.

4.4 Effect of treatment with guava leaf suspension and Doxycycline on the different blood parameters of quails infected with *E. coli*

Table 4 shows the results of different blood parameters in different treatment groups. In the present study, it was found that there was significant variation of TLC, neutrophil (%), Lymphocyte (%), eosinophil (%) among the different treatment groups (T_0, T_1, T_2 and T_3) but at day 22 and day 32, the eosinophil (%) varied insignificantly among the treatment groups. Similar results are also reported by El-Sayed *et al.,* (2013) who found that guava leaf significantly increased TLC count.

Table 4: Effect of treatment with guava leaf suspension and Doxycycline on the different blood parameters (TLC, Neutrophil, Lymphocyte and Eosinophil) of quails infected with *E. coli*

Age (Days)	T_0 (Mean±SEM)	T_1 (Mean±SEM)	T_2 (Mean±SEM)	T_3 (Mean±SEM)	Level of significance

TLC					
22	6600.00±70.71[b]	6000.00±70.71[a]	6800.00±70.71[b]	6200.00±70.71[a]	*
27	6500.00±70.71[b]	5800.00±70.71[a]	6600.00±70.71[b]	6000.00±70.71[a]	*
32	6200.00±70.71[a]	6700.00±70.71[b]	6500.00±70.71[b]	6200.00±70.71[a]	*
Neutrophil					
22	40.00±1.70[b]	50.00±1.70[c]	30.00±1.70[a]	40.00±1.70[b]	*
27	50.00±1.70[b]	60.00±1.70[c]	40.00±1.70[a]	50.00±1.70[b]	*
32	50.00±1.70[b]	45.00±1.70[a b]	40.00±1.70[a]	45.00±1.70[a b]	*
Lymphocyte					
22	20.00±1.61[a]	30.00±1.70[c]	25.00±1.84[a b]	25.00±1.84[a b]	*
27	30.00±1.70[a]	40.00±1.61[b]	35.00±1.61[a]	30.00±1.61[a]	*
32	60.00±1.70[b]	50.00±1.61[a]	45.00±1.70[a]	50.00±1.61[a]	*
Eosinophil					
22	2.00±0.32[a]	2.00±0.32[a]	1.00±0.32[a]	1.20±0.37[a]	NS
27	1.20±0.37[a]	3.00±0.32[b]	1.20±0.37[a]	1.20±0.37[a]	*
32	1.20±0.37[a]	2.00±0.32[a]	1.20±0.37[a]	1.20±0.37[a]	NS

Note: Values are expressed as mean± standard error of means. Means between column are statistically significant (P<0.05) *= Significantat 5% level of significance. NS means statistically not significant.

CHAPTER V
CONCLUSION AND RECOMMENDATION

This study was conducted to investigate the effect of guava leaf (*Psidium guajava*) suspension on *E. coli* quail. The treatment groups T_2, T_3 had statistically increased body weight and decreased bacterial load count in relation to control group. The mortality (%) was significant lower in T_2 group (%), which was higher in T_1 group that was infected with *E. coli*. It is concluded that guava leaf (*Psidium guajava*) suspension effectively controlled *E. coli* infection and had effective antibacterial activity. Guava leaf and Doxycycline had the more or less similar effect on the body weight and bacterial load. Guava leaf suspension can be used instead of Doxycycline. It is recommended that further research is needed to ascertain the mechanism of action for its application in clinical practice.

CHAPTER VI

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