

## Antibacterial Activity of Eight Medicinal Plants against Multidrug Resistant *Escherichia coli* and *Salmonella* spp. isolated from Broiler Meat

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### Abstract

The excess use of antimicrobial agents in the poultry industry is a significant reason for the gradual spread and increasing level of multidrug resistance bacteria. This article is based on a study in which the antibacterial activity of aqueous, methanolic, ethanolic and acetonetic extracts of eight medicinal plants were evaluated by standard disc diffusion method against multidrug resistant *Escherichia coli* and *Salmonella* spp. isolated from broiler meat. The multidrug resistance was checked by commercially available antibiotics using standard disc diffusion method. The results have indicated that the ethanolic extract of *Boerhaavia diffusa* showed maximum zone of inhibition against *Escherichia coli*, while *Asparagus racemosus* showed maximum zone of inhibition against *Salmonella* spp. Other experimental plant extracts had showed moderate activity against these multidrug-resistant bacteria, which can also be considered as potential source of active beneficial phytochemicals. Proper management and application of these plant extracts may be a wonderful alternative of commercially available antibiotic to minimize the risk.

### Keywords

Antibiotics; Broiler meat; Multidrug resistance; *Salmonella* spp.; *Escherichia coli*

## Introduction

Antibiotic resistance occurs when microorganisms can survive in the presence of an antibiotic that would normally inhibit their growth (Fadare *et al.*, 2019). Different kinds of antibiotics are used in the poultry industry to increase feed conversion, growth promotion, and disease prevention to improve meat production. Promoting growth in poultry production, antibiotics can be widely used successfully as sub-therapeutic, prophylactic, and growth-promoting agents, for nutritive purposes, and to protect the health of birds by modifying the immune status of broiler chickens (Hassan *et al.*, 2010; Emami *et al.*, 2012; Lee *et al.*, 2012; Chattopadhyay, 2014). This is mainly used to control the gastrointestinal infections and microbiota modification in the intestine of poultry birds (Torok *et al.*, 2011; Singh *et al.*, 2013). The mechanism remains unclear, but antibiotics are likely to act by remodeling microbial diversity and relative abundance in the intestine to provide an optimal microbiota for growth (Dibner and Richards, 2005). However, scientific evidence suggests that the massive use of these compounds has led to the increasing problem of antibiotic resistance and the presence of antibiotics residues in feed and environment compromises human and animal health (Furtula *et al.*, 2010; Forgetta *et al.*, 2012; Carvalho and Santos, 2016; Ronquillo and Hernandez, 2017). Due to the lack of knowledge, the condition and dosing rate of antibiotic in poultry farming is not proper and that may be the considerable reason for antibiotic resistance of different bacterial species. Indiscriminate use of antibiotics results into noticeable residues in meat, milk, cheese, butter, and other livestock products that may cause serious problems to consumers (Lee *et al.*, 2000).

Currently, it is estimated that antibiotic resistance causes approximately 700,000 people to die each year. By the year 2050, this number of deaths is predicted to reach 10 million annually (de Kraker, Stewardson and Harbarth, 2016). It represents one of the most alarming threats to global health, and new anti-infective agents are needed to overcome this antibiotic resistance (Thabit, Crandon and Nicolau, 2015). Hence, it is essential to find effective alternative agents to control infectious diseases and limit the spread of antibiotic-resistant bacteria. Further, new mechanisms of action of these novel anti-infective agents are needed (Schroeder, Brooks and Brooks, 2017). It is believed that plants may contain different bioactive compounds and that would be a good source of novel anti-infective agents (Rossiter, Fletcher and Wuest, 2017). In traditional medicinal practices against infections, plants have been used to inhibit the growth and virulence of various microbes (Ahmad and Beg, 2001; Kumar *et al.*, 2012; Bashir, Erum and Kausar, 2012, Cioch *et al.*, 2017). A diverse array of chemical compounds known as secondary metabolites are synthesized by plants to communicate with other organisms (Harborne, Baxter and Webster, 1994). In addition, these secondary metabolites are advantageous for anti-infective agents or bioactive drug development and harboring the potential for synergy with other secondary metabolites as a part of the plant's multicomponent defense system (Harvey, Edrada-Ebel and Quinn, 2015). There is no bacteriostatic or bactericidal medicine available in the market derived from plant secondary metabolites. Alternative anti-infective mechanisms of action can open new avenues in drug development to combat antibiotic resistance where natural products may serve as a critical reservoir to overcome resistance mechanisms (Schroeder, Brooks and Brooks, 2017; Wright, 2017). As there is a growing demand for alternative plant-derived compounds that have fewer side effects than synthetic compounds, and whose effectiveness can be improved by modern pharmacological methods (Wilasrusmee *et al.*, 2002). Based on observations of traditional use for the treatment of infectious diseases and literature searches on their reported known activities, eight medicinal plants were selected. These species are present in abundant populations in the collection regions (Tangail

district, Bangladesh) and none are listed as threatened or endangered. The objective of this study is to evaluate the antimicrobial activity of different extracts of eight medicinal plants against the growth of *E. coli* and *Salmonella* spp. The essential criteria for the selection of each plant species are explained below.

*Coccinia grandis* (Leaf): This plant belongs to the family of *Cucurbitaceae*. It is found in tropical Asia (India, Pakistan, Bangladesh, Sri Lanka, Indonesia, Malaysia, the Philippines, and Thailand), and Africa (Sakharkar and Chauhan, 2017). Various kinds of plant parts are valuable medicine and various preparations have been mentioned in the indigenous system of medicine for various skin diseases, bronchial phlegm and bronchitis. Traditionally, in Unani and herbal medicine used in treating worm, psoriasis, smallpox, scabies and other itchy skin eruptions and ulcers (Miller *et al.*, 2000; Quinlan, Quinlan, and Nolan, 2002). The effective leaves of the plant possess anti-diabetic, antipyretic, anti-inflammatory, analgesic, and antimicrobial properties. It is also useful to induce perspiration in fever and cures sores in the tongue (Farrukh *et al.*, 2008). Phytochemical screening of *C. grandis* reported the presence of saponins, cardenolides, flavonoids, and polyphenols may be attributed to antibacterial activity (Sivaraj *et al.*, 2011).

*Acacia macrorrhizos* (Stem): This is an important medicinal plant from the Araceae family in the tropical and subtropical regions of Asia, Sri Lanka, India, Bangladesh, China, South Africa, and mainly cultivated in India and Bangladesh (Mandal, Misra and Singh, 2010). It has various pharmacological properties like thrombolytic, antifungal, antimicrobial, anticancer, analgesic, hepatoprotective, hepatorenal. Its antioxidant property is also recommended in Ayurvedic text for prevention and treatment of inflammation of abdomen and spleen (Banik *et al.*, 2014). The juice of leaves is used as digestive, anthelmintic, laxative, diuretic, and astringent (Srivastava *et al.*, 2012). This plant contains flavonoids, oxalic acid, cyanogenic glycosides, alocaasin, cholesterol, amino acids, gallic acid, maleic acid, ascorbic acid, succinic acid, glucose, fructose, sucrose and beta lectins (Hasan and Sultana, 2018).

*Jatropha curcas* (Leaf): It was originated from Mexico with spread to Asia and Africa by Portuguese traders as a hedge plant. It belongs to the family of *Euphorbiaceae* (Rampadarath, Puchooa and Jeewon, 2016). Parts of *J. curcas* have been used in traditional medicine and in veterinary care (Suhaili *et al.*, 2011). *J. curcas* has shown characteristics of potent cytotoxic, anti-tumor, antimicrobial, and anti-fungal agent (Rahman *et al.*, 2014), it is also used as anticoagulant (Suhaili *et al.*, 2011). The extract of seeds and leaves of *J. curcas* have shown molluscidal and insecticidal properties (Rampadarath, Puchooa and Jeewon, 2016). It has also been used as antidote, remedy, medicine and a potential source of herbal drugs in dental complaints and against constipation. The milky sap is used for the treatment of dermatomucosal diseases (Dada, Ekundayo and Makanjuola, 2014). Moreover, *Jatropha curcas* (leaf) is commonly used for aching patients with colds or fever. A study has shown that it contains an alkaloid known as jatrophine, which is believed to have anti-cancerous properties. The latex of the plant can be used as a remedy for alopecia, anasarca, burns, dropsy, eczema, inflammation, paralysis and yellow fever (Morton 1980; Dada, Ekundayo and Makanjuola, 2014).

*Leucas lavandulifolia* (Leaf): *Leucas lavandulifolia* belongs to the family of Labiatae and is a well-known ethnomedicinal plant. It has been used in Bangladesh for traditional medicine from the time memorial and usually used in cough, cold, fever, loss of appetite, skin disease, headache, snakebite and scorpion sting (Makhija *et al.*, 2011). It also shows antibacterial activity against multidrug-

resistant bacteria such as *E. coli*. Other properties, i.e., hepatoprotective, hypoglycemic, antipyretic, antidiarrheal, antitussive, wound healing and psychopharmacological, antimicrobial activities of the plant have been reported by *in vivo* study conducted by Murugan, Mishra and Paul (2018).

*Boerhaavia diffusa* (Leaf): *Boerhaavia diffusa* belongs to the family of Nyctaginaceae. It is used as an Ayurvedic medicine in India and Unani medicine in Arab countries for the treatment of diabetes, jaundice, stress, dyspepsia, inflammation, abdominal pain, enlargement of the spleen and congestive heart failure (Akhter *et al.*, 2013). It is an herbaceous plant, cultivated in fields spreading vine widely distributed in tropical and subtropical regions (Sahu *et al.*, 2008). For medicinal purposes, the whole plant parts such as roots, leaves, flowers, seeds, etc. are used. Apart from above, *Boerhaavia diffusa* also possesses marked antimicrobial properties viz. antibacterial properties and antifungal properties (Olukoya, Idika and Odugbemi, 1993; Agrawal *et al.*, 2004; Aladesanmi *et al.*, 2007). It has also been reported to be useful in the treatment of elephantiasis, night blindness, corneal ulcers, and nephritic syndrome (Umamaheswari, Nuni and Shreevidya, 2010). All the properties have made this plant very important in the treatment of human and plant diseases.

*Catharanthus roseus* (Leaf): *Catharanthus roseus* belongs to the family of Apocynaceae, which is an erector procumbent herb or undershrub containing latex. Different studies have shown that *Catharanthus roseus* contains more than seventy different types of therapeutic agents or alkaloids that can be used in treating various diseases including lung cancer, breast cancer, uterine cancer, melanomas and Nonhodgkin's lymphoma (Kainsa, Kumar and Rani, 2012). It is reported that the plants have antibacterial potential in crude extracts of different parts (viz., leaves, stem, root, and flower) (Muhammad *et al.*, 2009). It possesses known antibacterial, antimicrobial, antifungal, and diabetic, anticancer and antiviral activities against numerous cell types (Raza *et al.*, 2009).

*Hemidesmus indicus* (Root): *Hemidesmus indicus* (root) is pharmacologically important plant belonging to the *Asclepiadaceae* family (Kavitha *et al.*, 2010). A study showed that *H. indicus* root extract inhibited *Salmonella typhimurium* induced pathogenesis nonspecifically, by reducing hydrophobicity of bacterial cell surface and perhaps also by mimicking host cell receptors and thereby blocking its attachment to host cell (Das and Devaraj, 2006). The *H. indicus* root powder or its water extract can increase its antidiarrheal efficacy if incorporated in oral rehydrating salt solution (Evans, Rajasekharan and Subramoniam, 2004.). The plant root is said to be tonic, as its root decoction helps in skin diseases, elephantiasis, syphilis, blood purification, loss of appetite and for kidney and urinary disorders. Other biological activities i.e., antidiabetic, antioxidant, hepatoprotective, antithrombotic, anti-ulcerogenic, anti-inflammatory, immunomodulatory, etc. have been reported from *Hemidesmus indicus* root extracts (Ratha *et al.*, 2012).

*Asparagus racemosus* (Root): This plant belongs to the family of Aspragaceae. It is an important medicinal plant traditionally used as anthelmintic, antiseptic, antidiarrheal, and antidysentery and anti-cancer agent (Kunwar *et al.*, 2009). This plant is recommended in Ayurvedic medicine for prevention and treatment for inflammation, nervous disorder, liver diseases, infectious diseases, gastric ulcers, dyspepsia, and as a galactagogue (Sinha and Biswas, 2011). The methanolic root extract of *A. racemosus* is beneficial to *in vitro* antibacterial activity against various common pathogens (Mandal *et al.*, 2000). The root of *Asparagus racemosus* has shown considerable activity

against nervous disorders, dyspepsia, diarrhea, dysentery, inflammations, neuropathy, hyperdipsia, antioxidant, antitussive, and certain infectious diseases (Goyal, Singh and Lal, 2003).

## Materials and Methods

### *Study area*

This study was conducted in 5 regions of Sherpur district, Bangladesh (figure 1) during the period from March 2017 to December 2017. In this area, poultry rearing has become very popular, though the environment is unhygienic. It was an important reason for evaluating the antibiogram pattern of isolates where all the samples were randomly selected and collected from poultry farms or local retail markets.



Figure 1: Geographical location of study area

### *Collection and transportation of samples*

A total of 50 dressed broiler carcasses were collected. From among them 25 were collected during winter and 25 were collected during the summer season. They were immediately brought to Microbiology Laboratory of the Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Tangail through maintaining cool chain using icebox.

### *Preparation of samples for bacteriological studies*

Each of the raw meat samples (thigh muscle, breast muscle, and drumstick) was macerated in a mechanical blender using a sterile diluent as per the recommendation of the International Organization for Standardization (Hossain *et al.*, 2015). Ten grams of the thigh meat sample was taken aseptically with sterile forceps and transferred into sterile containers containing 90 ml of 0.1% peptone water. A homogenized suspension was made in a sterile blender. Thus, 1:10 dilution of the samples was obtained.

### *Isolation of associated bacteria*

The primary culture was performed in nutrient agar and nutrient broth media. For sub-culturing, suspected bacteria were inoculated separately into different bacteriological media under the aseptic condition and incubated at 37°C for 18 hours. Pure cultures were achieved by further sub-culturing on selective agar.

### *Identification of associated bacteria*

Cultural, morphological, and biochemical characteristics were studied to identify the bacterial flora. The cultural characteristics or colonial morphology of the bacterial grown on MacConkey agar, SS agar, the eosin methylene blue (EMB) and xylose lysine deoxycholate (XLD) agar were recorded. Gram staining was performed to study the morphology and staining characteristics of the bacteria. Biochemical tests, such as sugar fermentation, methyl red (MR), Voges-Proskauer (VP), and indole tests, were performed to identify the bacteria tentatively. Isolates were confirmed by microscopic, cultural and standard biochemical tests (KIA, motility, catalase, coagulase, oxidase, urease, citrate utilization, indole, gelatin hydrolysis, MR-VP, TSI test) according to Bergey's Manual of Determinative Bacteriology (9th Edition, 1994) for further analysis (Bullock and Aslanzadeh, 2013).

### *Collection and preparation of plant extract*

The leaves, stems, and roots of the desired plants were collected from the university campus of Mawlana Bhashani Science and Technology University. All the plant parts were washed with clean water to remove dirt, dried in air and pulverized using mortar and pestle. The plant extracts of different concentrations were made in different solvents using a vacuum evaporator machine by following standard method (Zrostlikova *et al.*, 2002).

### *Antibiotic Susceptibility Testing*

The antibiotic susceptibility of the isolates i.e., *Salmonella* and *E. coli* was determined using the standard disc-diffusion method (Adesiyun *et al.*, 2007). The antibiotic discs (Oxoid®, UK) used in this study were: ampicillin (10 µg), azithromycin (15 µg), amoxicillin (10 µg), bacitracin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), cefaclor (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), nalidixic acid (30 µg), norfloxacin (10 µg), penicillin G, (10 units), streptomycin (10 µg), sulphomethoxazole tetracycline (30 µg), vancomycin (30 µg).

### *Evaluation of the antibacterial activity of plant extract by disk diffusion method*

The disk of filter paper was made and soaked in different concentrations of plant extract and dried for the use of susceptibility test of *Salmonella* and *E. coli*. The disks soaked with water, ethanol, methanol and acetone were used as a control disk (Tambekar *et al.*, 2009).

## Results and Discussion

### *Biochemical Test for Bacterial Analysis*

Two kinds of bacteria (*E. coli*, *Salmonella* spp.) were isolated by observing distinct morphological characteristics on selective media and further confirmed with standard biochemical tests (Table 1). The *E. coli* isolates were observed in EMB agar as smooth, circular, greenish-black color colonies with metallic sheen whereas pink color colonies were observed in Mac-Conkey agar (Schroeder, Brooks and Brooks, 2017; Wright, 2017). The *Salmonella* spp. were observed as smooth, circular, black centered colonies in XLD agar and SS agar. In Gram's staining, the morphology of both bacteria exhibited Gram-negative, short rod arranged in single or paired (Wilasrusmee *et al.*, 2002; Schroeder, Brooks and Brooks, 2017; Sakharkar and Chauhan, 2017).

Table 1: Biochemical test for bacterial analysis

Gram Staining	Biochemical reaction															Presumptive Bacteria	
	EMB plate	KIA			MIU			Simon' s Citrate	VP test	Oxidase	Catalase	Mannitol	Starch hydrolysis	Methyl Red	Glucose		Lactose fermentation test
		Slant	Bud	Gas	Motility	Indole	Urease										
-Ve	+	A	A	+	+	+	+	-	-	-	+	A	-	+	A G	+	<i>E. coli</i>
-Ve	-	K	A	G	+	-	+	+	-	-	-	A	-	+	-	-	<i>Salmonella</i> spp.

### *Bacterial load of Broiler Meat during Winter and Summer Season*

Bangladesh has six seasons: summer (Grisma), rainy (Barsa), autumn (Sarat), late autumn (Hemonta), winter (Shhit), spring (Basanta). Each season stays normally for two months. However, three seasons, pre-monsoon, rainy monsoon or summer and winter season, are prominently recognized. Winter comes after the late autumn, and, generally, it starts in October and lasts up to February. Winter temperature ranges from 3°C or 6 °C to 10 °C. On the other hand, summer season stays from March to June at a temperature range between 25°C to 35°C. Total Viable Count (TVC), Total Coliform Count (TCC), Total *E. coli* Count (TEC), Total *Salmonella* spp. Count (TSC) of samples were conducted among six places of Nalitabari township during winter and summer season (Figure: 2). Microbial count is slightly higher in all the samples compared to the standard. The

favorable environmental conditions of summer and unfavorable acclimatization of winter are an important factor for this high microbial load in summer. *Shigella* was not found in any samples that were collected in either summer or winter season.

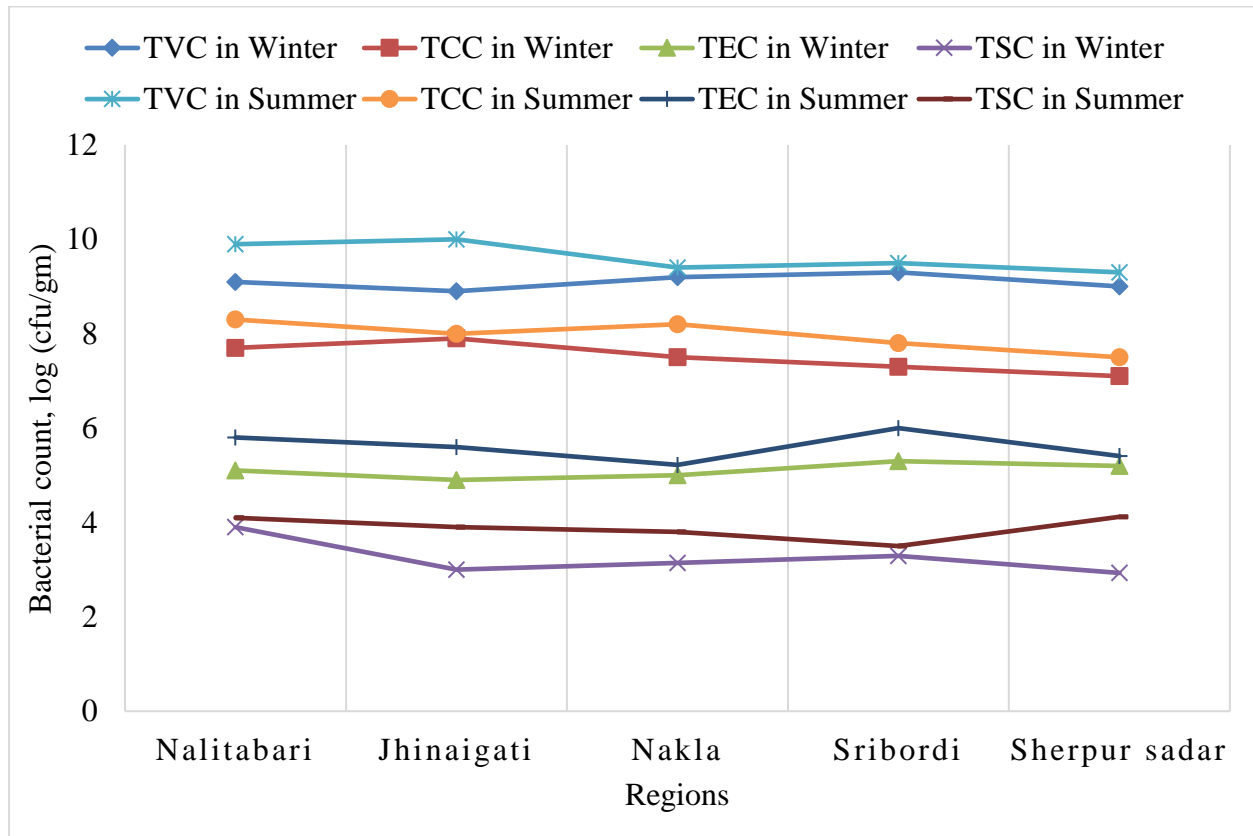


Figure 2: Presence of microorganism in boiler meat during winter and summer sessions

#### Antibiotic Resistance Pattern

The possession of such factors by the *E. coli* isolates signifies the fact that the organisms might have gained the resistance property due to the indiscriminate use of antibiotics. The occurrence of isolated bacteria should be considered as hazardous to health and advocate the preventing risk factors. Resistance against commercially available and commonly used antibiotics has been observed in bacteria present in broiler since the introduction of these antimicrobial agents in poultry discriminately. The rise in antibiotic resistance has been reported in the past two decades in many countries including Bangladesh (Saha *et al.*, 2005). Antibiotic susceptibility test was done in randomly selected 30 isolates (15 of *E. coli* and 15 of *Salmonella* spp.). The response of commercially available antibiotics against bacteria was categorized into three groups, i.e., sensitive, intermediate, and resistant. In this study, *E. coli* showed high resistance to Amoxicillin (93.33%), Ampicillin (86.67%), Chloramphenicol (80%), Penicillin (80%), 60% resistance to Streptomycin and Erythromycin, and 53.33% resistance to Neomycin.



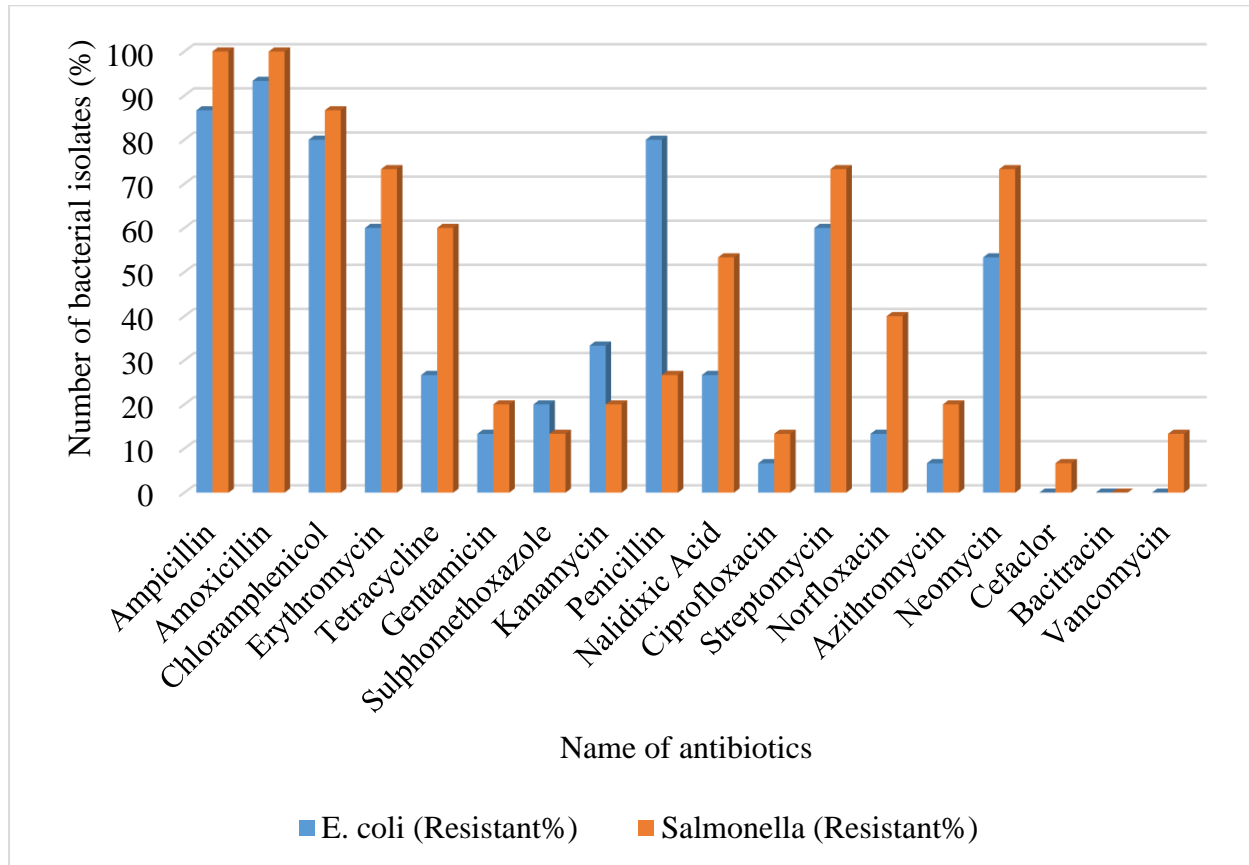


Figure 3: Antibiogram profiles of *E. coli* and *Salmonella* spp.

*E. coli* has shown high sensitivity to Bacitracin (93.33%), Vancomycin, (86.67%), Cefaclor (80%), Ciprofloxacin (80%), Gentamicin (73.33) and Azithromycin. (53.33%) (figure 3). *E. coli* was moderately sensitive to Tetracycline (53.33%) and Norfloxacin (60%). The results strengthen the earlier observation of other studies (Perry and Metzger, 1980; Behl and Srivastava, 2002; Schroeder, Brooks and Brooks, 2017). The resistance of *E. coli* was observed against Ampicillin, Amoxicillin, Chloramphenicol, Penicillin, Erythromycin and Streptomycin (Schroeder, Brooks and Brooks, 2017). Earlier observations of studies conform to the fact that the *E. coli* isolated from broiler were sensitive to Azithromycin, Ciprofloxacin, Norfloxacin, and Gentamicin and resistant to Amoxicillin and Erythromycin. The possessions of such factors by the *E. coli* isolates signify the fact that the organisms might have gained the resistance property due to the unsystematic use of antibiotics (Chetri *et al.*, 2020).

*Salmonella* spp. were 100% resistant to antibiotics Ampicillin and Amoxicillin, 60% resistant to Tetracycline and 53.33% resistant to Nalidixic Acid (figure 3), but they were highly sensitive to Chloramphenicol (86.67%), Bacitracin (80%), Erythromycin (73.33%), Gentamicin (73.3%), Ciprofloxacin (73.3%) and Vancomycin (73%). Previous studies also revealed that *Salmonella* spp. were sensitive to Ciprofloxacin, Gentamicin, and Azithromycin (Manjunath, Prakash and Vamseedhar Annam, 2011; Ansari *et al.*, 2014) and resistant to Erythromycin and Amoxicillin (Nesa, Khan and Alam, 2011; Mulatu, Beyene and Zeynudin, 2014). Potential drug-resistant pathogens in normal broilers may be a serious public health concern. It was revealed that *Salmonella* spp. were sensitive to Bacteriocin, Vancomycin, Ciprofloxacin, Gentamicin, and

Azithromycin (Mandal *et al.*, 2010; Schroeder, Brooks and Brooks, 2017) and resistant to Chloramphenicol, Streptomycin, Neomycin and Erythromycin (Schroeder, Brooks and Brooks, 2017).

#### *Evaluation of Antibacterial Activity of Eight Medicinal Plants against Multidrug Resistant E. coli and Salmonella spp.*

Extract of eight different plants was used for antibacterial study against two different isolates such as *E. coli* and *Salmonella* spp. Ten *E. coli* and ten *Salmonella* spp. isolates were selected on the basis of antibiogram study, where they were resistant to more than one commercially used antibiotics (*E. coli* was higher resistance to Amoxicillin, Ampicillin, Chloramphenicol, Penicillin, when *Salmonella* spp. were maximum resistant to antibiotics Ampicillin and Amoxicillin, Chloramphenicol and Erythromycin). Four solvents such as double distilled water, methanol, ethanol and acetone were used for the preparation of plant extract. Commercially available Ciprofloxacin antibiotic was used as positive control.

#### *Aqueous Plants Extracts against E. coli and Salmonella spp.*

Aqueous extract of six plants including *C. grandis*, *A. macrorrhizos*, *L. lavandulifolia*, *C. roseus*, *H. indicus* and *A. racemosus* did not exhibit any activity at any concentration against *E. coli*. *J. curcas* exhibited zone of inhibition (9 and 8 mm) at two higher concentrations (i.e., 100 and 50 mg/ml) and *B. diffusa* has showed zone of inhibition (11, 10, 8 and 7 mm) at four concentrations (i.e., 100, 50, 25, 12.5 mg/ml) where both plants did not express any activity at the lowest (6.25 mg/ml) concentration (Figure 4).

Aqueous extract of *C. grandis*, *A. macrorrhizos*, *L. lavandulifolia*, *C. roseus*, *H. indicus* and *A. racemosus* did not exhibit any zone of inhibition against *Salmonella* spp. *J. curcas* exhibited a zone of inhibition (8 mm) at only 100 mg/ml concentration. *B. diffusa* has shown a zone of inhibition (10, 9, 7 mm) at four concentrations (i.e., 100, 50, 25, 12.5 mg/ml).

Aqueous extract *C. grandis* and *J. curcas* (100 mg/ml) did not show any activity against *E. coli* but *J. curcas* (100 mg/ml) showed activity against *Salmonella* (11.7 mm) as demonstrated by a previous study (Suhaili *et al.*, 2011). Aqueous extract of *A. macrorrhizos* did not show any activity against *Salmonella* but has showed activity against *E. coli* (Farrukh *et al.*, 2008). Aqueous extract of *B. diffusa* leaves showed higher activity against Gram-negative bacteria and moderate antibacterial activity against *E. coli* and *Salmonella* (Umamaheswari, Nuni and Shreevidya, 2010).

#### *Antibacterial Activity of Methanol Extracts against E. coli and Salmonella spp.*

Methanolic extract of eight plants exhibited a favorable result against *E. coli*. *A. racemosus* exhibited maximum activity (18, 16, 15, 12 and 10 mm) against *Salmonella* in all experimental concentrations (100, 50, 25, 12.5, 6.25 mg/ml). *A. macrorrhizos* and *J. curcas* exhibited 14 mm zone and *B. diffusa* and *C. roseus* exhibited 17 mm zone of inhibition at the highest concentration (100 mg/ml) where all of these four plants expressed 7 mm zone of inhibition at a lowest concentration (6.25 mg/ml). *C. grandis* (10 mm), *L. lavandulifolia* (13 mm) and *H. indicus* (11 mm) expressed a moderate activity at highest concentration against *E. coli*. The last two of these plants did not express any activity at the lowest concentration.

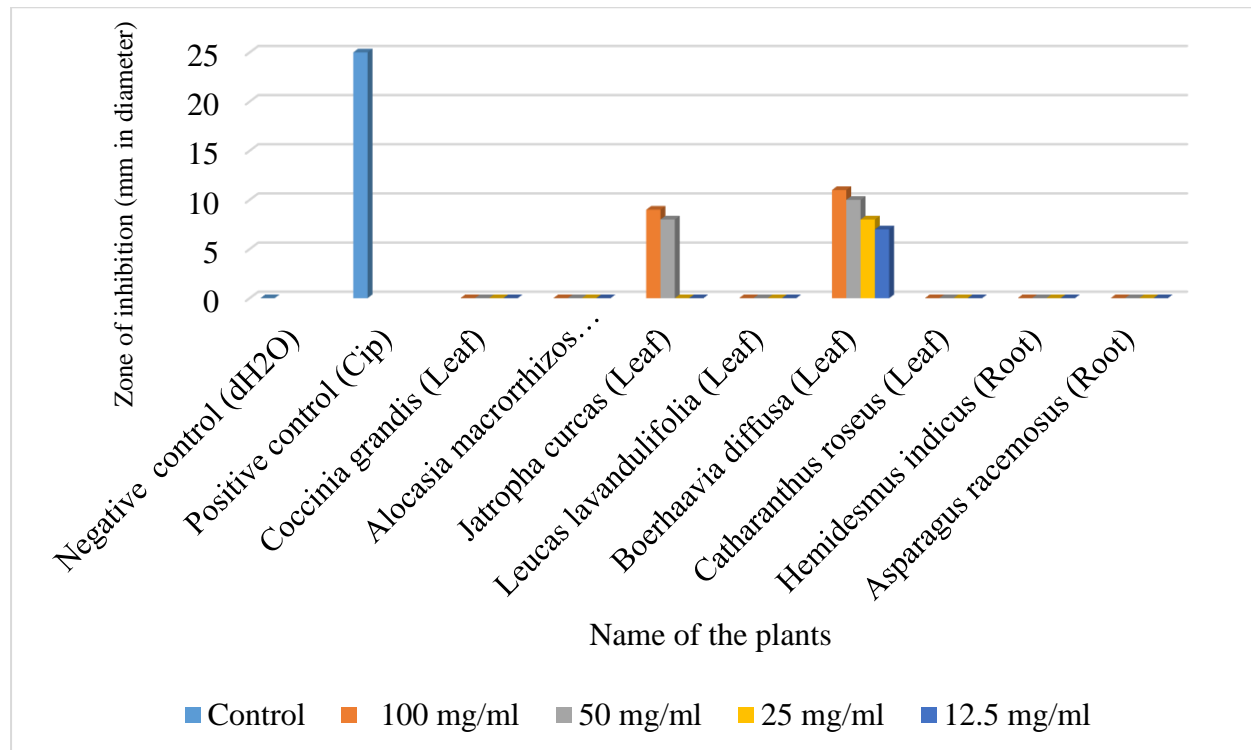


Figure 4: Antibacterial activity of aqueous plants extracts against *E. coli* [Cip = Ciprofloxacin]

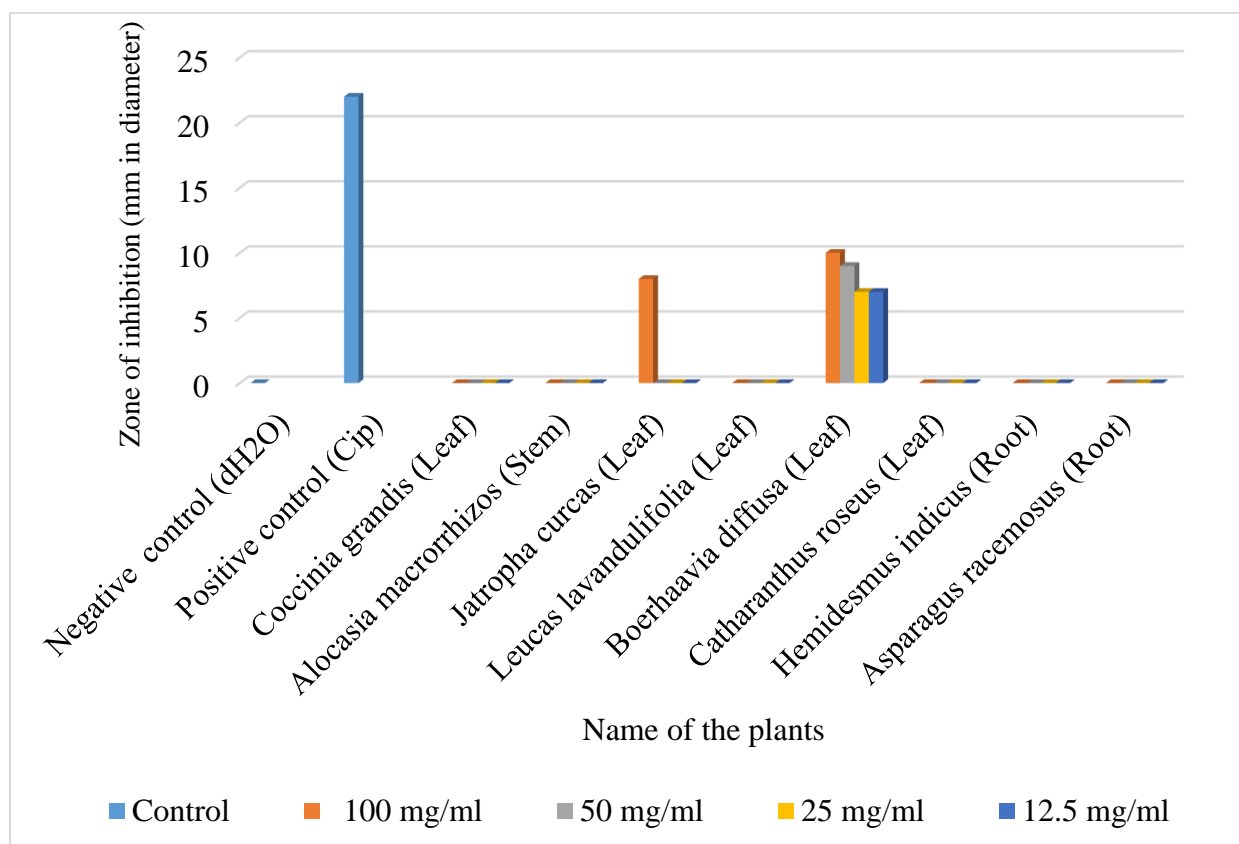


Figure 5: Antibacterial activity of aqueous plants extracts against *Salmonella* spp. [Cip= Ciprofloxacin]

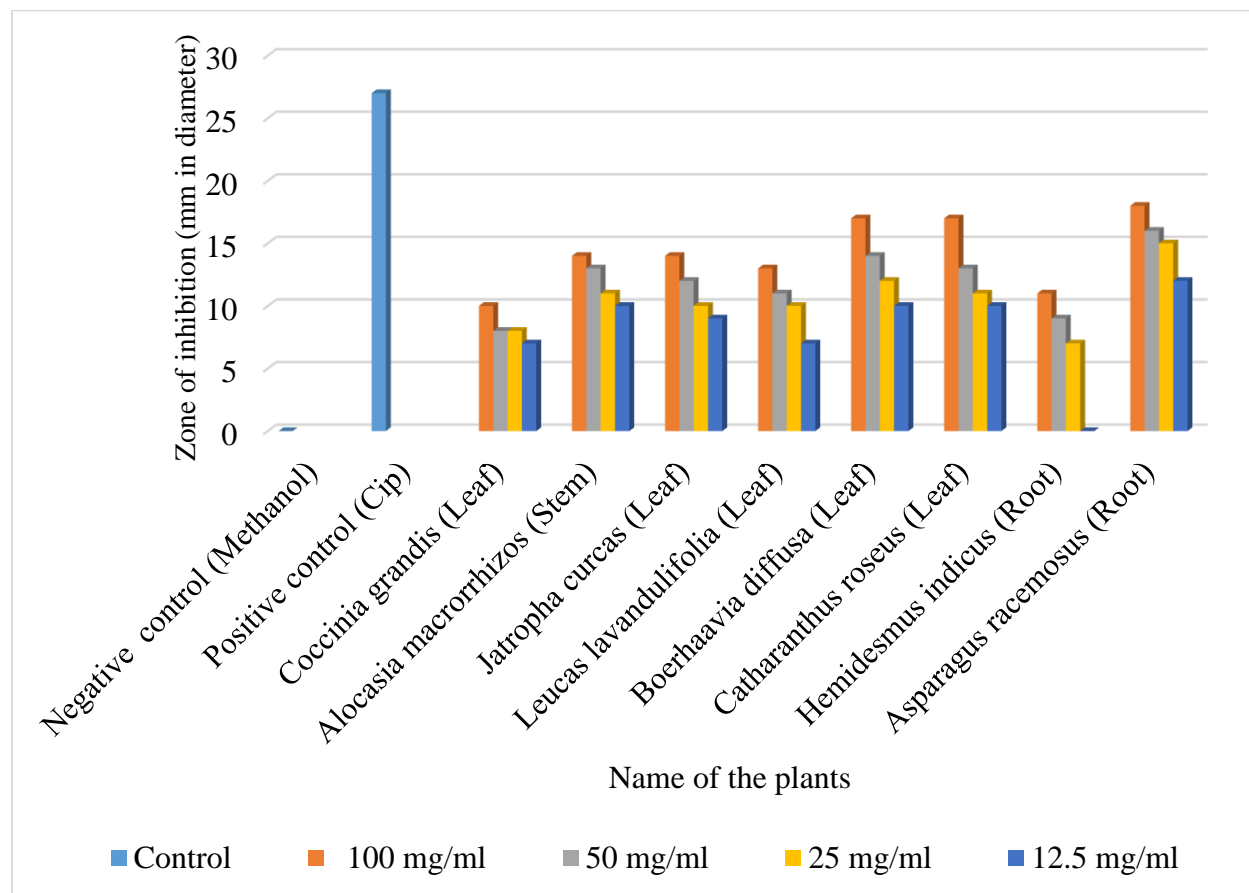


Figure 6: Antibacterial activity of methanol extracts against *E. coli* [Cip = Ciprofloxacin]

The antibacterial activity of *A. racemosus* methanolic extract was highest among these eight plants extract against *Salmonella* at five experimental concentrations. The plant extract of *C. grandis* and *L. lavandulifolia* did not have any antibacterial activity against *Salmonella* in any concentration. The activity of *A. macrorrhizos* (10 mm), *J. curcas* (12 mm), *B. diffusa* (14 mm), *C. roseus* (13 mm) and *H. indicus* (12 mm) were moderate at a highest concentration, whereas the activity of last three plants was found in all concentrations.

The zone of inhibition of methanol extract of *A. macrorrhizos* and *H. indicus* (root) was 9.1 mm and 8.5 mm against *Salmonella*, whereas it was 7.5 mm and 9 mm against *E. coli* (Farrukh *et al.*, 2008; Ratha *et al.*, 2012). Previous data described the zone of inhibition of *J. curcas* (latex) methanol extract was 12.3 mm and 15 mm at 100 mg/ml against *E. coli* and *Salmonella* and did not find any activity of *L. lavandulifolia* (leaf) against both the microbes (Suhaili *et al.*, 2011; Murugan, Mishra and Paul, 2018). A previous study demonstrates the methanolic extract of *A. racemosus* (root) has high antibacterial activity against *E. coli* and *Salmonella*, and *B. diffusa* did not show any activity against *E. coli* and *Salmonella* (Umamaheswari, Nuni and Shreevidya, 2010). Methanol extract of *C. roseus* leaf showed antibacterial activity against *E. coli* and *Salmonella* that was about 10 mm and 6 mm, respectively. The root extract showed highest activity, which was 10 mm and 24 mm (Raza *et al.*, 2009).

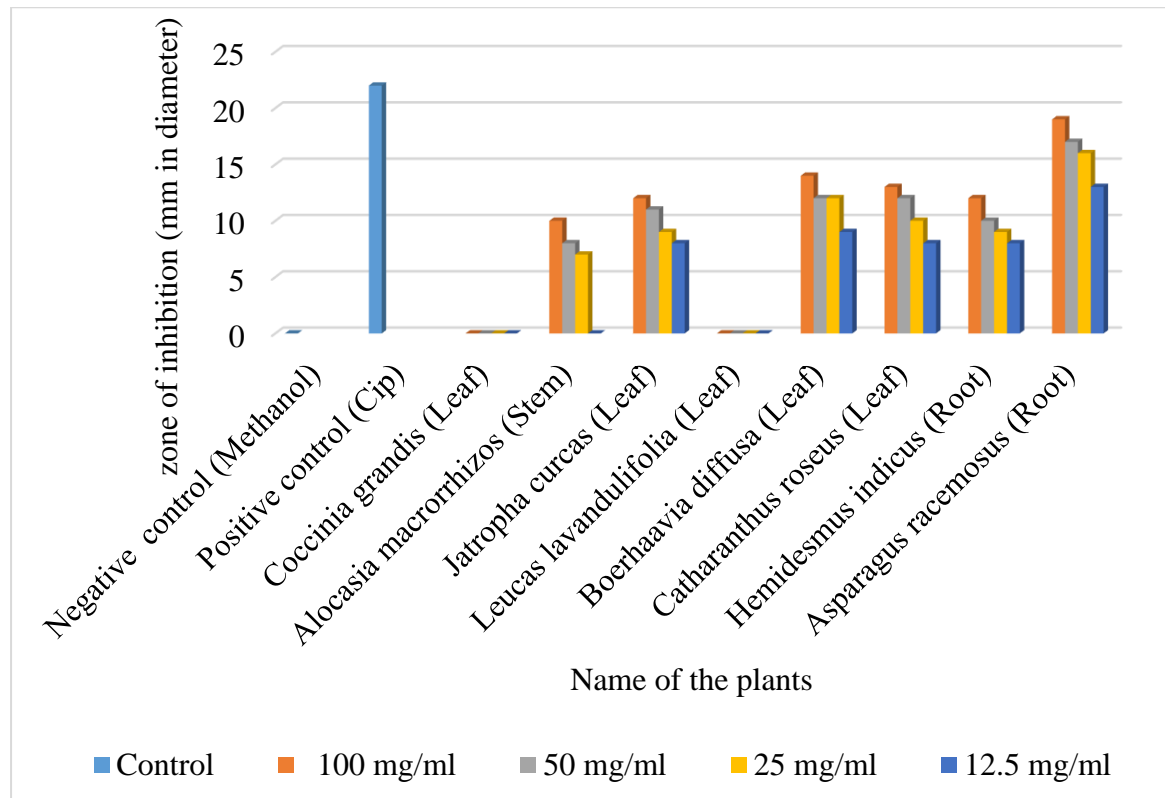


Figure 7: Antibacterial activity of methanol extracts against *Salmonella* spp. [Cip = Ciprofloxacin]

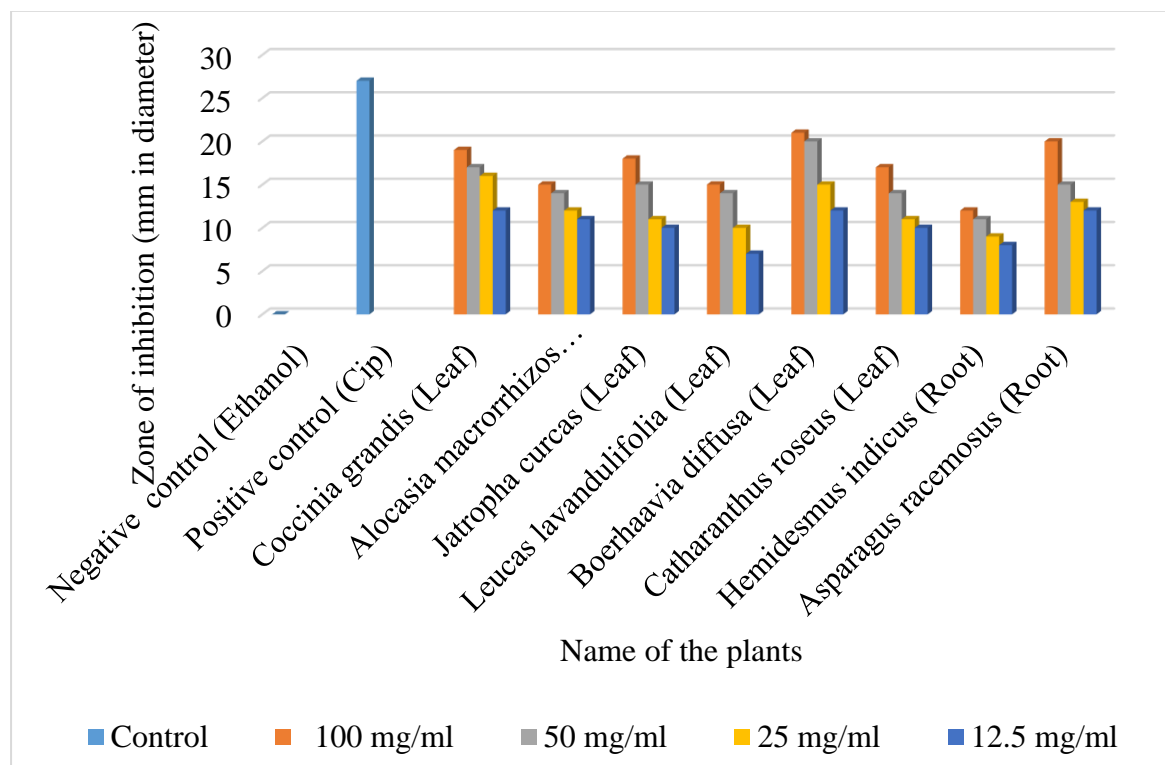


Figure 8: Antibacterial activity of ethanol extracts against *E. coli* [Cip = Ciprofloxacin]

*Antibacterial Activity of Ethanol Extracts against E. coli and Salmonella spp.*

The activity of ethanolic extract of eight plants in all concentration was recorded against *E. coli*. The ethanolic extract of *B. diffusa* expressed maximum zone of inhibition (21, 20, 15, 12 and 10 mm) in all concentration against *E. coli*. The activity of *A. racemosus* (20, 15, 13, 12 and 9 mm) was similar to that of *B. diffusa*. The ethanolic *C. roseus* (17 mm), *J. curcas* (18 mm), *C. grandis* (19 mm) and *H. indicus* (12 mm) exhibited moderate activity at maximum concentration against *E. coli*, whereas the activity of *H. indicus* and *L. lavandulifolia* plants extracts was same (15 mm) at highest concentration.

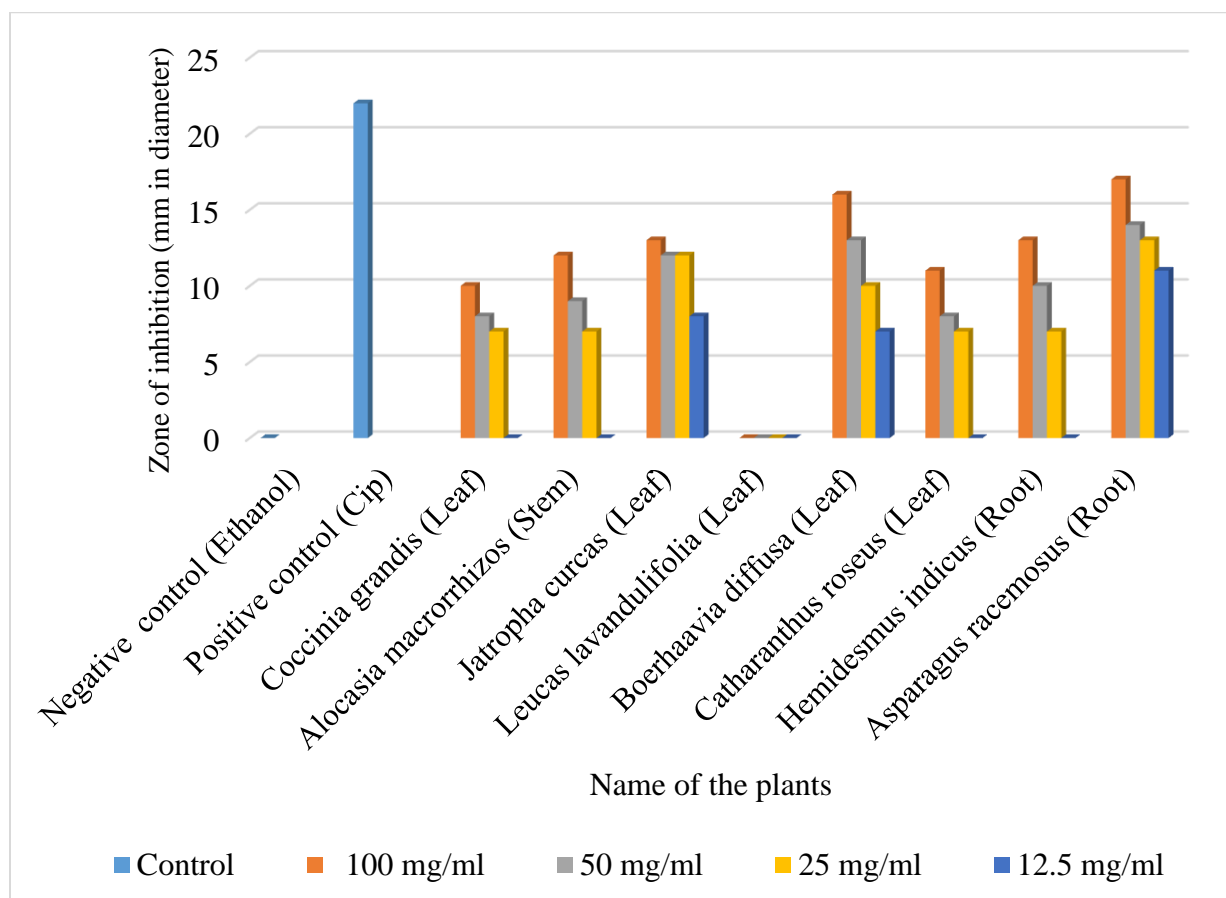


Figure 9: Antibacterial activity of ethanol extracts against *Salmonella* spp. [Cip = Ciprofloxacin]

Ethanolic extract of *A. racemosus* showed maximum activity (17, 14, 13, 11, and 9 mm) in all concentrations (100, 50, 25, 12.5 and 6.25 mg/ml), and *L. lavandulifolia* did not show any activity against *Salmonella*. The highest concentration of *J. curcas*, *B. diffusa*, *C. grandis*, *A. macrorrhizos*, *C. roseus* and *H. indicus* showed 13, 16, 10, 12, 11 and 13 mm zone of inhibition, while last four of these plants did not show any activity against *Salmonella* in lowest two concentrations (12.5 and 6.25 mg/ml).

The zone of inhibition of ethanol extract of *C. grandis* (stem) was 6.6 mm against *Salmonella* and 6 mm against *E. coli* as showed in the earlier study (Farrukh *et al.*, 2008). The data of the past study demonstrated that ethanol extract of *B. diffusa* leaves showed more activity against a Gram-

negative bacterium and strongly supports the high antibacterial activity of *A. racemosus* against *E. coli* and *Salmonella* (Umamaheswari, Nuni and Shreevidya, 2010). Earlier experiments demonstrated that ethanol extract of *H. indicus* (root) expressed their activity against *E. coli* and *Salmonella* were 11 mm and 8.5 mm, respectively (Ratha *et al.*, 2012). The previous study supports the finding that ethanol extract of *A. racemosus* (root) has similar activity against those microbes (Samy, Ignacimuthu and Sen, 1998).

#### Antibacterial Activity of Acetone Extracts against *E. coli* and *Salmonella spp.*

Acetone extract of *L. lavandulifolia* (16, 15, 13, 13 and 12 mm) showed maximum activity in all concentrations (100, 50, 25, 12.5, 6.25 mg/ml) against *E. coli*, whereas the activity of *C. grandis*, *A. macrorrhizos*, *B. diffusa* and *H. indicus* plants did not show the activity in lowest two concentrations (12.5 and 6.25 mg/ml). The zone of inhibition of *C. roseus* (15 mm), *J. curcas* (14 mm), *A. racemosus* (13 mm), *A. macrorrhizos* (10 mm), *H. indicus* (9 mm) and *C. grandis* (8 mm) gradually decreased compared to the zone of inhibition of *L. lavandulifolia* in maximum concentration (100 mg/ml).

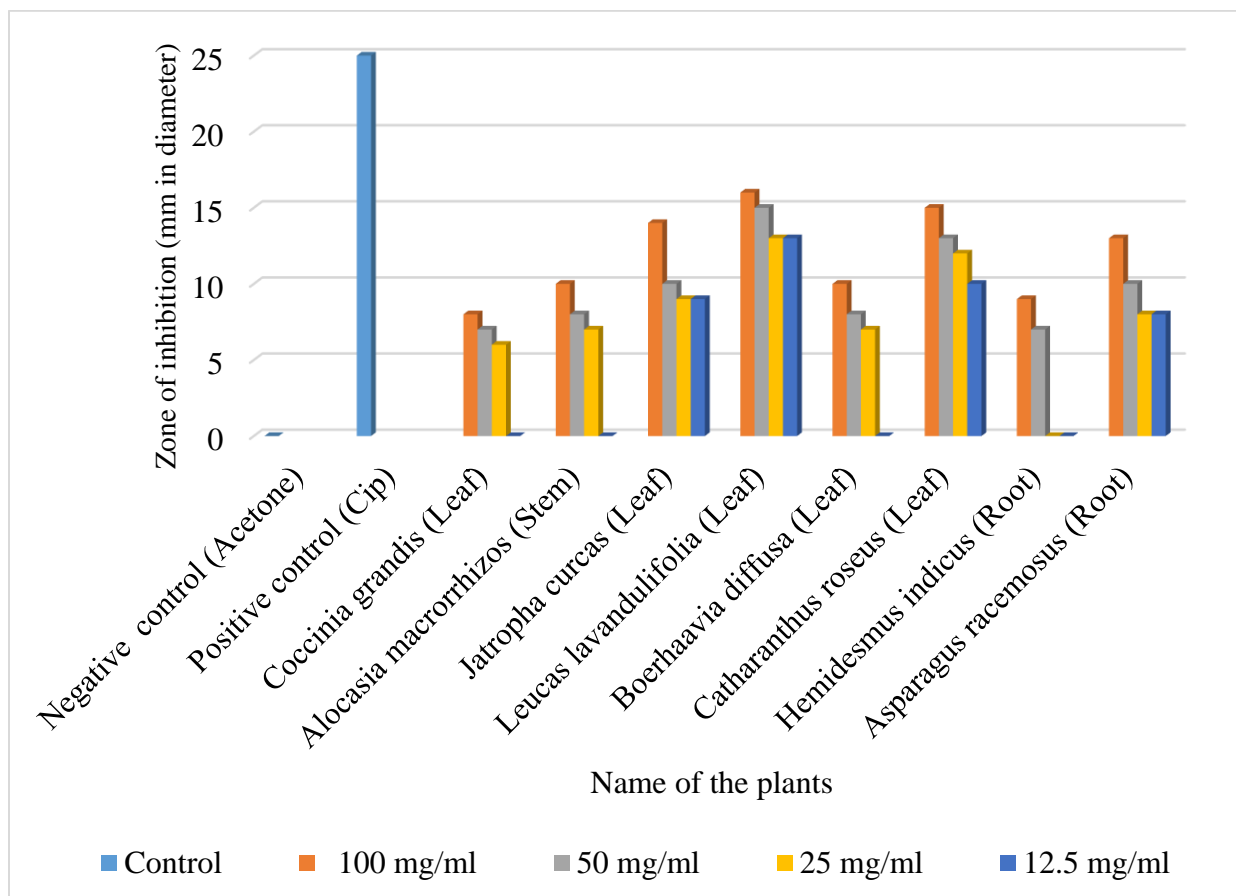


Figure 10: Antibacterial activity of acetone extracts against *E. coli* [Cip = Ciprofloxacin]

Acetone extract of *A. racemosus* showed maximum activity (14, 13, 10 and 7 mm) against *Salmonella spp.* in the first four concentrations, whereas the lowest concentration of all plants did not show any type of activity. The activity of *J. curcas* (10 mm) and *C. roseus* (11 mm) was

moderately high and the zone of inhibition of *B. diffusa*, *A. macrorrhizos* and *H. indicus* showed comparatively small (8 mm) zone of inhibition against *Salmonella* at 100 mg/ml concentration. Acetonic extract of *C. grandis* and *L. lavandulifolia* did not show any activity in any concentration against *Salmonella*. In an earlier study, it was found that *A. racemosus* (root) showed moderate zone of inhibition against *E. coli* and *Salmonella* (Ahmad, Mehmood and Mohammad, 1998).

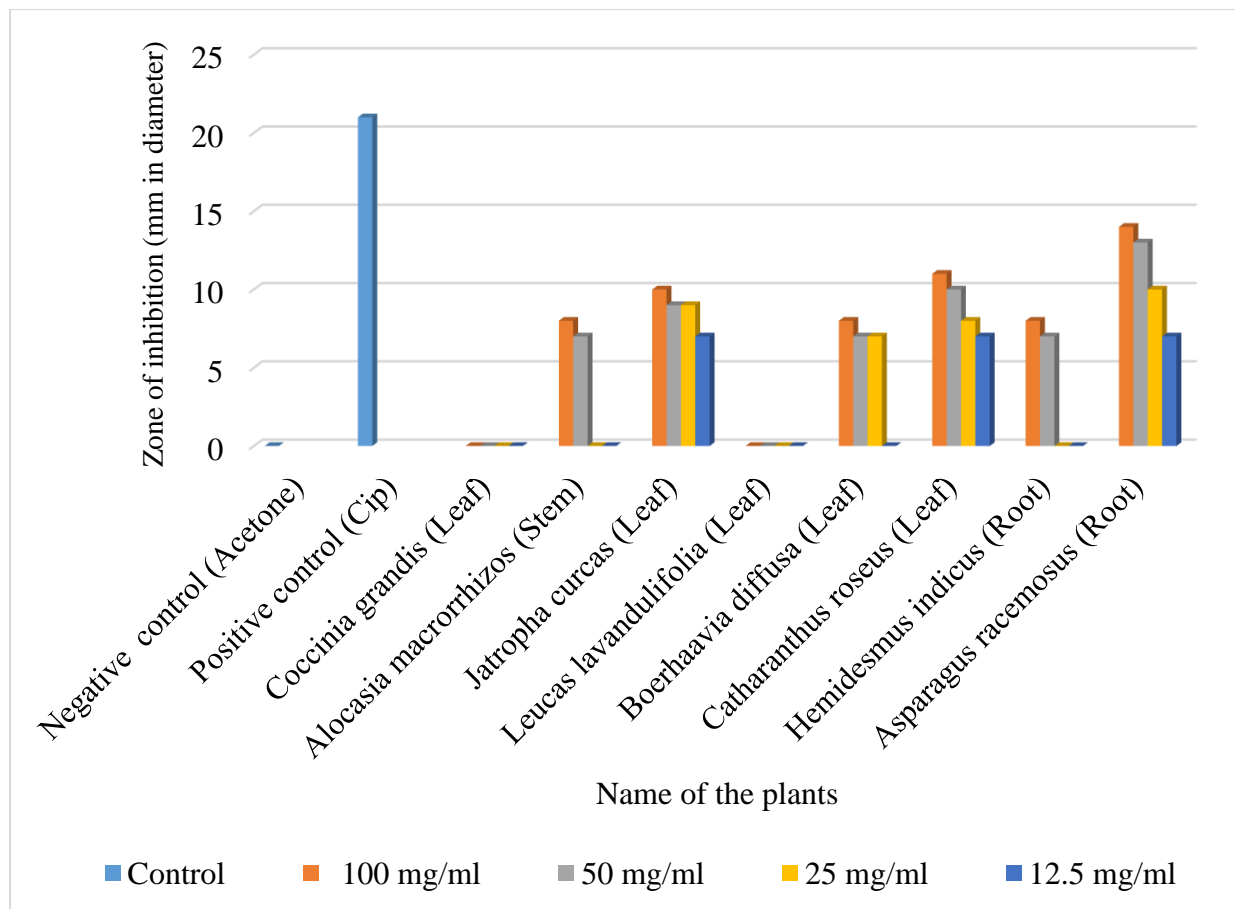


Figure 11: Antibacterial activity of acetone extracts against *Salmonella* spp. [Cip = Ciprofloxacin]

## Conclusion

Antibiotic resistance has limited the use of antibiotics against infectious microbes. Plant extract which contains phytochemical or secondary metabolites like alkaloids, flavonoids etc. can play an essential role that can block the activity of resistant microbes. The present study showed that the tested eight medicinal plants in the form of aqueous and organic solvent extracts at different concentrations have a positive effect against multi-drug resistant *E. coli* and *Salmonella* spp. Thus, it can be concluded that these eight medicinal plants (i.e., *Coccinia grandis*, *Alocasia macrorrhizos*, *Jatropha curcas*, *Leucas lavandulifolia*, *Boerhaavia diffusa*, *Catharanthus roseus*, *Hemidesmus indicus*, *Asparagus racemosus*) can be used as a supportive therapy along with the standard antibiotics to treat infectious diseases in broiler chickens.



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