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Antimicrobial Activity Of *Euphorbia Turicalli* And *Aloe Vera* Against Avian Pathogenic *Escherichia Coli*.

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APPROVAL FORM

Title of the research project: **ANTIMICROBIAL ACTIVITY OF *EUPHORBIA TURICALLI* AND *ALOE VERA* AGAINST AVIAN PATHOGENIC *ESCHERICHIA COLI*.**

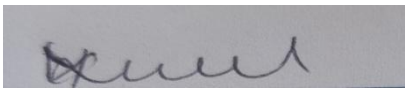
The undersigned certify that they have read the project and it is suitable for submission to the Facultyof Science and Engineering, and checked for conformity with the Faculty.

Supervisor:  Date: 01/10/2024

Chairperson:  Date: 04/10/24

DECLARATION

I, **YEMURAI KAMUZONDE(B202511B)** declare that this research is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned author(s) either electronically or otherwise.

Signed: 

Date: 06/06/24

SUPERVISOR

I ……J. Ndava……. declare that I have supervised this research project and I am satisfied that it can be submitted to the Faculty of Science and Engineering of Bindura University of Science Education.

Signature:  Date:. 01/10/2024

DEDICATION

This project is dedicated to my supportive parents Mr. B and Mrs. B Kamuzonde and sisters, Kudzai and Kundai Kamuzonde

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Above all I give thanks to the Almighty God whom I cannot do without.

ABSTRACT

The rising incidence of antibiotic resistance among avian pathogenic *Escherichia coli* (APEC) strains poses a significant threat to poultry health and the poultry industry. In response, there is an increasing interest in exploring plant-based antimicrobial agents as potential alternatives to conventional antibiotics. The aim of the study was to compare the antibacterial activity of *Euphorbia turicalli* and *Aloe vera* extracts on avian pathogenic *Escherichia coli* (APEC). The Agar well diffusion method was used to evaluate the antibacterial activity of each of the crude extracts. Extracts for *Euphorbia turicalli* reflected antibacterial inhibitory properties against *E. coli*. The methanol extract at a concentration of 75% had the largest inhibition zones against the avian *E. coli* strain with a mean diameter of 7±0.0mm. The largest mean zone of inhibition for the acetone extract against *E.coli* was 3.7 ±0.14 mm. The largest zone of inhibition for *Euphorbia turicalli* aqueous extract identified was 2.2±0.0mm. A significant difference was observed in the inhibition zone means for methanol, acetone and aqueous *Euphorbia turicalli* extracts (p=0.03). The *Euphorbia turicalli* methanol extract at a concentration of 75% had the largest inhibition zones against the avian *E. coli* strain with a mean diameter of 9.5mm. The largest mean zone of inhibition for the acetone extract against *E.coli* was 7.7±0.23mmmm. The zone of inhibitions noted after treatment with different concentration of the water extract were relatively small compared to those of methanol and acetone extracts. The largest inhibition zone for aqueous extract was 4.6±mm at a concentration of 4.6 mm. There was a significant difference in the mean inhibition zones. There were significant differences in the antibacterial effects of *Euphorbia turicalli* and *Aloe vera* extracts against avian pathogenic *E.coli* (APEC) (p=0.03). The most effective extract was the methanol *Aloe vera* extract with an MIC of 6.25mg/ml. For both methanol, aqueous and acetone extracts *Aloe vera* extracts were the most effective with MIC values of 6.25mg/ml, 18.5mg/ml and 12.25mg/ml respectively. The study recommends further research on the efficacy of *Euphorbia turicalli* and *Aloe vera* on other resistant strains like MRSA. The study concludes that both extracts from *E. tirucalli* had antibacterial properties against *E. coli.* Zones of inhibition and MIC values found for *E. tirucalli* extracts suggest that the species was highly effective and might be applied to the management of illnesses brought on by the avian pathogenic *E. coli.*

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LIST OF ABBREVIATIONS

MAC: MacConkey agar

MIC: Minimum Inhibitory Concentration

RV: Rappaport-Vassiliadis soya peptone broth

TBX: Tryptone Bile X-glucose

XLD: Xylose lysine deoxycholate

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CHAPTER 1

INTRODUCTION

1.1 Background to the Study

Colibacillosis is the aggregate term for infections in poultry caused by avian pathogenic *Escherichia coli* (APEC). According to Nolan *et al.,* (2013), this particular *E. coli* type may possess virulence characteristics that allow it to spread extra intestinally, leading to significant financial losses and compromised animal welfare in industrial poultry production. Prior to 2013, Nolan *et al.,* (2013) noted that APECs were primarily thought of as secondary pathogens that needed predisposing factors like compromised skin or mucosal barriers, other infections, an impaired mononuclear phagocytosis system, immunosuppression from viral infections and toxins, poor ventilation, and poor hygiene in order to cause infections. Sequence types (ST) 95 and 117 of *E. coli* have been linked to APEC epidemics in the Nordic region. This suggests that *E. coli* plays an essential role rather than a secondary function in the infections (Ronco *et al.* 2017).

The identification of ST117 as the predominant clone in broiler cellulitis further implies that APEC has an important function in causation of the disease (Poulse *et al.,* 2018).According to Olsen *et al.* (2012), *E. coli* infections in recently hatched chickens can begin as yolk sac or navel infections, both of which are linked to septicemia. Eventually, *E. coli* infection may enter through the respiratory system, cuts, wounds, and/or organs which release eggs. Airsacculitis, polyserositis, and even sepsis may occur after respiratory tract invasion. According to Jorda *et al.* (2005), Olse *et al.* (2016), and Thøfner *et al.* (2019), the infection known as salpingitis or salpingitis and peritonitis begins with an ascending infection through the cloaca and may cause septicemia.

According to Chantziara *et al.* (2017), the colonization of hens with antibiotic-resistant APEC strains is influenced by the strain's initial prevalence, antibiotic treatment, including the mode of delivery, and the strain's suitability. It was recently discovered that broilers are extremely vulnerable to ESBL or ampC-producing APE infection. The preventive use of third-generation cephalosporin in hatcheries was, for example, found to increase the frequency of *E. coli* isolates becoming resistant to that type of antibiotics when compared to untreated chicks. It has been found that the use of antibiotics selects for antibiotic-resistant *E. coli* (clonal dissemination). Furthermore, even in

the absence of antibiotics, antibiotic-resistant genes may still be passed from one bacterium to another, including APEC. Nonetheless, the exposure to drugs will raise the prevalence (Argu-din *et al.*, 2017).

Worldwide interest in using herbal remedies as a source of antibacterial medicines is growing significantly. The majority of plants and their parts contain phytoconstituents with a wide range of properties, including antimicrobial, anti-inflammatory, antioxidants, antipruritic, hypotensive, proliferative, hypoglycemic, and analgesic properties that are essential to wound healing (Blanca, 2006). Many medicinal plants have been screened for possible antimicrobial properties due to the rise in chemotherapeutic failure, antibiotic resistance shown by harmful infectious pathogens, and the emergence and reemergence of infectious diseases (Kumar *et al.* 2015). Because of these properties and the ease with which plants can be obtained, they are frequently used in ethnoveterinary medicine. Knowledge in ethnoveterinary medicine is a collection of beliefs and practices regarding animal welfare that involves the use of natural resources (plant and animal) and other materials. Typically passed down orally from generation to generation, this knowledge is currently threatened, along with other traditional beliefs, by technological advancements, sociocultural shifts, and environmental changes. (Blanca, 2006).

Commercial drugs have become prohibitively expensive for low-income smallholder farmers in Zimbabwe, making them an unaffordable resource for managing livestock health at small scale. Owners do, however, possess a thorough understanding of ethnobotany, which serves as the foundation for identifying medicinal plants that may be used to make veterinary and medical medications. Smallholder farmers with limited resources have always used medicinal plants as a form of therapy for their livestock (Marume, *et al.*, 2017). Recent years have seen a notable increase in research efforts aimed at comprehending the effectiveness of ethnoveterinary techniques in managing microbiological entities that impact humans, animals, and livestock (Wanyama, 1997).

Among these plants is *Euphorbia turicalli.* The succulent plant *Euphorbia turicalli,* sometimes referred to as pencil cactus or milk bush, has a long history of usage in medicine, ornamental gardening, and industrial settings (*Wal et al. 2013*).*Euphorbia turicalli* is a small tree or unarmed succulent shrub that grows up to 5 meters tall. Its brittle juicy branches are around 7 millimeters thick and green with fine white striations running along them. According to Curtis and Mannheimeir, (2005), leaves are linear, lenceolote to narrowly abovate, freshly present only on new growth, and rapidly deciduous. The Ndebele term for it is ingotsa, while the Shona name it hejiyemukaka/rusungwe. In Zimbabwe, it is often planted as a poisonous hedge that is resistive to goats, particularly on communal lands. According to Riina *et al.* (2013), *Euphorbia turicalli* is widely distributed in tropical Africa, the Arabian Peninsula, Madagascar, India, and the Far East. It is well known that the plant's milky sap has anti-inflammatory, anti-cancer,

and antimicrobial effects. It has been stated that several cultures in Brazil, India, Indonesia, and Malaysia have conducted studies on *Euphorbia turicalli* in order to treat various health conditions, including warts, asthma, cough, cancer, malignancies, and dental pain. Studies on ethnomedicine have revealed that Euphorbia turicalli is a plant with significant ethnomedical value that has been very helpful to the traditional healers in the area. Research revealed that bioactive chemicals found in *Euphorbia turicalli* exhibit antibacterial action against *Candida tropicalis, Aspergillus niger, Escherichia coli,* and calbicans (Mavundza *et al.,* 2022).

The other most cited plant that is seems to poses antimicrobial properties is *Aloe vera*. The succulent cactus plant aloe vera has triangular, fleshy leaves that are green with white teeth on the edges. It often reaches a height of 60 to 100 cm. The aloe plant is native to Africa and few offshore islands. It is the only plant in the genus Aloe. Southern Africa is home to more than 150 species of aloe, while Zimbabwe is home to more than 30 species. *Aloe vera* is known by the Shona name gavakava and the Ndebele name icena. For generations, aloes have been used medicinally. *Aloe vera* products are often used to treat constipation, herpes sores, acne, wounds, and flea bites. It is important to remember, nevertheless, that although *aloe vera* is thought to be safe for usage in tropical climates, consuming *aloe vera* can have negative side effects, such as cramping in the abdomen and diarrhea (Guo & Mei, 2016). According to recent studies in Zimbabwe, *A.vera* and *Euphorbia turicalli* has been found to be one of the most used herbs in rural poultry management. For small scale poultry farmers the purchases of convectional drugs have become very expensive and it has proved to be out of reach for them. Therefore there is need to maximize the use of plants like *Aloe vera* for the heath care of their poultry. None of these aforementioned plants have been assessed for their efficacy against pathogenic *E. coli* affecting chicken in Zimbabwe.

The poultry business is affected by colibacillosis, which is caused by *Escherichia coli* and threatens flocks of chicken globally, leading to a high mortality rate (Dziva & Stevens, 2008). It poses a risk to the lives and habitats of the livestock. The poultry industry is searching for alternatives to antibiotics that can be used to treat infections brought on by avian pathogenic *E. coli*. Third- and fourth-generation cephalosporins must not be used on animals for food. This seems to be particularly important for hens where antibiotics are automatically injected into the feed and water in significant volumes. According to *Saidi et al.* (2013), strains of avian pathogenic *E. coli* are starting to show resistance to the approved antibiotics that are used to treat them. Poultry farmers have resorted to using herbs as medicine for their sick birds.

Poultry farmers have turned to using herbs as medicine for their sick birds. It is recommended that Zimbabwe follow the lead set by the US broiler industry, which has transitioned to never using antibiotics for the majority of its output. Research on treating sick birds with alternative therapies, such plant extracts, can help achieve this.

Extracts from the *Aloe vera* plant have been mentioned as one of the plants that Zimbabwean farmers have utilized to treat sick birds. To increase the variety of plants that farmers can employ to treat avian pathogenic E. coli, additional studies on other plants is required. Thus, the antibacterial activity of plant extracts from *Euphorbia turicalli* and *Aloe vera* will be examined in this study against the avian pathogen *E. coli*.

1.2 Problem Statement

The avian pathogenic *Escherichia coli* (APEC) poses a significant threat to poultry health and productivity globally, causing avian colibacillosis which leads to substantial economic losses in the poultry industry due to mortality, reduced egg production, and compromised meat quality (Kathayat *et al.,* 2021). In 2012 the prevalence of colibacillosis in Zimbabwe was at 48% (Saidi *et al.,* 2012). Previous studies indicated that antibiotic resistance related to colibacillosis in Zimbabwe is on the rise (Mbanga *et al.,* 2015). This therefore, shows that there is need for research on new antimicrobial agents. Alternative antimicrobial medicines produced from natural sources are becoming more and more necessary as antibiotic-resistant bacteria continue to arise (Belén et al., 2012).

1.3 Justification

Traditional antibiotic treatments are becoming less effective due to the emergence of antibiotic-resistant strains, necessitating the exploration of alternative antimicrobial agents derived from natural sources. In order for pharmaceutical companies to create natural veterinary and human medicine, more research on the therapeutic value of herbal medicine needs to be conducted and published hence validating the importance of the current study. The study's findings will give the Zimbabwe Department of Veterinary Services documented information that will enable them to determine whether or not farmers should utilize ethno veterinary medicine like *Euphorbia turicalli* and *Aloe vera* in addition to currently existing veterinary practices to help the eradicate disease.

1.4 Aim of the Study

To determine the antibacterial activity of *Euphorbia turicalli* and *Aloe vera* extracts on Avian pathogenic *E.coli* (APEC)

1.3.1 SPECIFIC OBJECTIVES

- To evaluate the antibacterial activity of extracts of *Euphorbia turicalli* extracts against avian pathogenic *Escherichia coli* (APEC)
- To evaluate the antibacterial activity of extracts of *Aloe vera* extracts against avian pathogenic *Escherichia coli* (APEC)
- To compare the antibacterial effects of *Euphorbia turicalli* and *Aloe vera* extracts against avian pathogenic *Escherichia coli* (APEC)
- To determine the minimum Inhibitory concentrations for Euphorbia turicalli and Aloe vera against E. coli.

1.5 Research Questions

- Can *Euphorbia turicalli* extracts inhibit the growth of avian pathogenic *Escherichia coli* (APEC)?
- Can *Aloe vera* extracts inhibit the growth of avian pathogenic *Escherichia coli* (APEC) Is there a difference in the antibacterial activity of *Euphorbia turicalli Aloe vera* extracts against avian pathogenic *Escherichia coli* (APEC) ?
- What are the minimum Inhibitory concentrations for *Euphorbia turicalli* and *Aloe vera* against avian pathogenic *Escherichia coli* (APEC)?.

1.6 RESEARCH HYPOTHESIS

H_O : *Aloe vera* and *Euphorbia turicalli* have no antibacterial effects against avian pathogenic *E.coli* (APEC).

H_1 : *Aloe vera* and *Euphorbia turicalli* have antibacterial effects on avian pathogenic *E.coli* (APEC).

1.7 Limitations of study

Initially, the absence of uniformity in the extraction procedure could result in variations in the makeup and amount of active ingredients, which could influence the noted antimicrobial properties. Furthermore, the study's dependence on a small number of APEC strains might have an incomplete representation of the variety within the bacterial population and how it reacts to the extracts. Furthermore, because the study was conducted in vitro, it is unable to take into consideration the intricate interactions that take place within a living creature, which could result in the essential aspects that affect the antibacterial activity in vivo being overlooked. Also, the absence of information on the extracts' pharmacokinetics in the study restricts our comprehension of their consumption, delivery, metabolic processes, and elimination in avian hosts. Finally, it might be difficult to evaluate the extracts' therapeutic relevance because the study does not compare the extracts' effectiveness to traditional antibiotics that are frequently used against APEC.

1.8 Delimitations

The study prioritized particular plant extracts or portions, therefore disregarding other plant parts that might have distinct antibacterial characteristics. This boundary limits the evaluation to the chosen extracts and can miss significant contributions from various plant constituents. Only APEC strains will be evaluated, leaving out any analysis of how the extracts affect different strains of bacteria. This restriction limits the findings' applicability to one specific bacterium. Furthermore, the research will solely utilize in vitro tests, so ignoring the intricacy of in vivo interconnections and any differences in efficacy. This constraint limits the ability to comprehend how well the extracts work in practical situations. Additionally, the study might overlook the long-term impacts and possible development in favor of concentrating on the short-term antibacterial effects.

1.9 Definition of terms

Antimicrobial Activity

Antimicrobial activity is defined as an agent's capacity to either stop the growth of microorganisms, including bacteria, viruses, fungi, and parasites, or to eradicate them. It is a gauge of how well a material works to stop or manage microbial illnesses. Many techniques can be used to evaluate the antibacterial activity, such as in vitro studies, in which the material is directly exposed to microorganisms in a controlled laboratory environment. The

outcomes of these experiments reveal details on the substance's range and potency of action against particular kinds of microbes.

Euphorbia turicalli

Euphorbia turicalli is a species of succulent plant in the Euphorbiaceae family. It is often referred to as the pencil cactus or milk bush. Originating from Africa, this plant is grown as an ornamental in many parts of the world.

Aloe vera

Aloe vera is a species of succulent plant that is a member of the Asphodelaceae family and the Aloe genus. Although it originated in the Arabian Peninsula, it is currently grown all over the world for a variety of therapeutic and cosmetic purposes. Aloe vera, sometimes known as the "true aloe" or "medicinal aloe," has a long history of traditional use.

The thick, fleshy leaves of the plant are arranged in a rosette-like pattern. Succulent and verdant, these leaves contain a transparent gel-like material that is often used for its medicinal qualities. Numerous bioactive substances are included in the gel, such as vitamins, minerals, amino acids, polysaccharides, and enzymes.

Minimum Inhibitory Concentration

The lowest concentration of an antimicrobial agent that prevents the apparent development of a microbes, such as yeast, bacteria, or viruses, is known as the Minimum Inhibitory Concentration (MIC), and it is measure used in microbiology and pharmacology. The (MIC) test is a useful tool for assessing how well antimicrobial drugs work against microorganisms.

CHAPTER 2

LITERATURE REVIEW

2.1 Ethno veterinary Medicine

Traditional knowledge is in danger of disappearing since it has been passed down orally through generations in many nations where it has not been well documented. The scientific term for traditional animal health care is ethno-veterinary medicine. Ethno veterinary medicine includes the knowledge, skill sets, techniques, practices, and beliefs that people of a community have for animal health care. The body of knowledge varies not only across and within societies, but also between regions. It was created by purposeful experimentation and trial and error. As a result, it is not as organized, methodical, or widely accepted as an effective means of controlling animal disease. Though they are less effective in treating and managing endemic and epidemic infectious diseases like septicemia, anthrax, and acute life-threatening bacterial diseases, traditional healers can manage a reasonable range of common ailments like diarrhea, wounds, colds, worms, coccidiosis, and reproductive disorders (Blanca, 2006).

Excellent ethno botanical knowledge is possessed by livestock owners, and this information serves as the foundation for identifying botanical components that may be used to make therapeutic drugs. Using indigenous cures, the herders of Kenya's Turkana and Samburu clans classified roughly sixty ailments in cattle as treatable or incurable. Of these, about thirty-five were curable, including mange, cough, diarrhea, and streptothricosis (Blanca, 2006). Additionally, scientific studies and trials conducted by farmers in Trinidad and Tobago discovered that enhancing drinking water with plant preparations like *Normadica charantia* increases broiler productivity and profitability. Goats have responded well to the use of paw-paw latex (*Cicaria papaya*) as an anthelmintic medication (Agbabiaka, 2008).

Despite these achievements, ethno veterinary knowledge has not found a place in the mainstream of veterinary practice, and very little of this traditional knowledge has been documented in developing nations. However, local veterinary practices and ethno veterinary knowledge have received more attention in recent years (Maroyi, 2017). Some of these techniques are becoming recognized as therapeutic, and it is essential to record them now before this information is lost. Issues in Zimbabwe's modern veterinary health system increase the need to conserve an increasingly endangered ethno veterinary heritage. Scarcity, unpredictable supply, and exorbitant cost limit the availability of veterinary health services and pharmaceuticals. Despite the existence of a vast network of veterinary hospitals, livestock owners are forced to treat their animals themselves, seek advice from a local healer, or slaughter the animal if the cost of care exceeds a substantial portion of the animal's worth due to a lack of manpower and a

poor communication infrastructure. Traditional healers receive payment in kind from satisfied customers rather than receiving a fee for their services. Given these limitations, it becomes crucial to look for alternatives. There is much room for growth in ethno veterinary medicine, which also offers an affordable substitute for allopathic drugs. (Zakeri & Kashefi, 2012).

2.1.1 Aloe vera

For ages, people have grown *Aloe vera* , a tropical and subtropical plant or herb, for its therapeutic and medical benefits (Kafshdouzan *et al.,* 2013). The two main sources of fluids in *A. vera* are yellow latex and clear gel (mucilage) made by massive parenchymatic cells in the leaf (Saidi *et al.,* 2013). It is indigenous to Northern Africa and is the most well-known species in the Aloe genus, which have approximately 500 species and are members of the Aloaceae family. AV leaf cross-section is shown in Fig. 1. Many studies have found that *A. vera* has a wide range of biological activities, such as anti-inflammatory, antioxidant, immunological modulatory, and cell growth boosting qualities (Zakeri & Kashefi, 2012). It also has antibacterial, antiviral, and antifungal characteristics. Additionally, other characteristics have been documented, such as anti-inflammatory and antibiotic effects against particular diseases (diabetics, cancer, allergies, and AIDS) (Eshun *et al.,* 2004). According to Aburjai *et al.* (2004), *A. vera* gel is mostly used in the beauty industry for wound healing, burn treatment, and scar treatment. Jasso de Rodriguez *et al.* (2005), have reported on the antifungal activity of A. vera gel against a variety of pathogenic fungi, including *Botrytis cinerea*. The use of *A. vera* gel as a functional ingredient in the Ethno veterinary medicine has drawn more attention lately (Moore *et al.,* 2005).

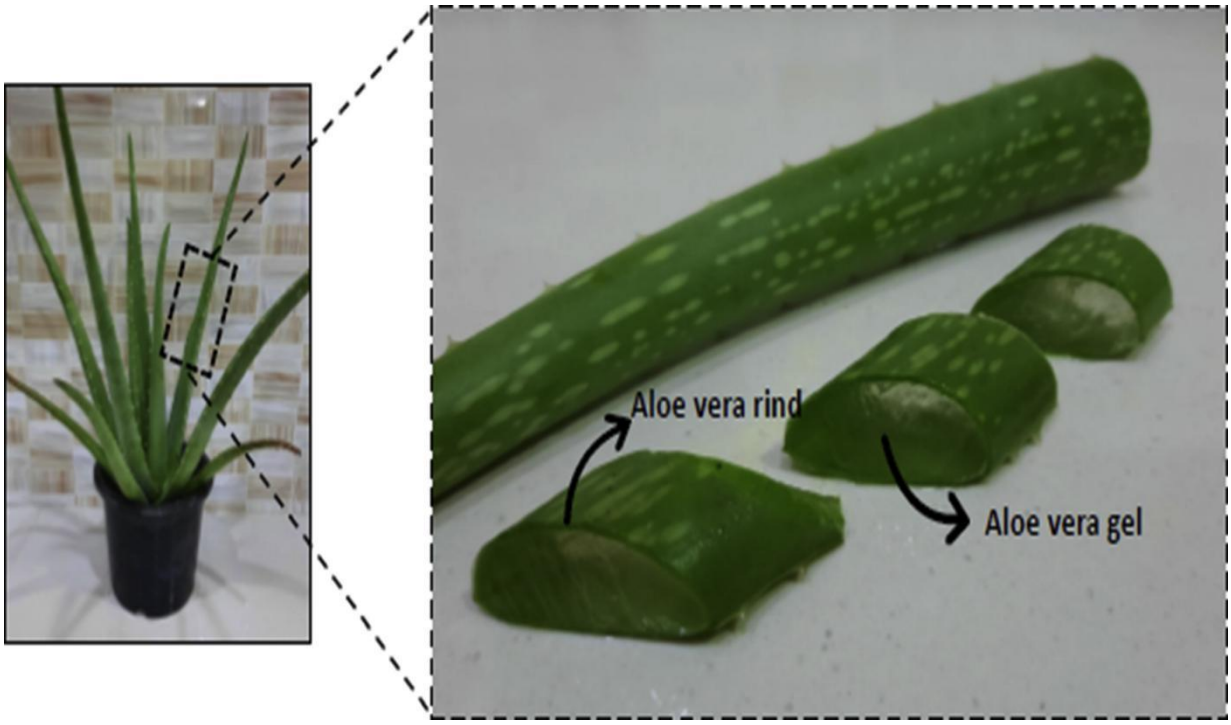

Figure 1*: Aloe vera plant* (Source: Niazim *et al.*, 2021)

2.1.2 *Euphorbia turicalli*

Euphorbia turicalli, frequently referred to as Tetulang, is a member of the Euphorbiaceae family and one of the most significant trees in the world, valued for its many applications. According to Mwine and Van Damme, (2011), it is a tiny tree or evergreen shrub that is native to tropical regions and has pencil-like branches that exude white latex. Many studies have shown that this plant is a good source of pharmacological substances. Studies revealed that the active ingredients in *Euphorbia turicalli*, such as phenols, tannins, and alkaloids, were what made the plant useful as a medicine (Sugumar *et al.* 2010). Researchers discovered that while phenolic and polyphenols were poisonous to microorganisms, flavonoids in *Euphorbia turicalli* efficiently prevented the growth of the bacteria by forming a compound with extracellular proteins of the cell wall and disrupting the microbial membrane.

Upadhyay *et al.* (2010) discovered that *Euphorbia turicalli's* tannins have the ability to inhibit bacteria by deactivating enzymes, cell membrane transport proteins, and bacterial adhesion. Euphorbias have historically been used as folk remedies for cough, asthma, rheumatism, cancer, and other conditions. *Euphorbia turicalli's* stem is used to treat splenic infections, asthma, and whooping cough. It has been reported that the alcoholic extract of

Euphorbia turicalli's stem exhibits broad-spectrum antibacterial action against *Salmonella enteritidis, Escherichia coli, Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus* and *S. epidermidis*.

According to Jadhav *et al. (*2010), the alcoholic extract of *Euphorbia turicalli's* stem exhibited broad-spectrum antimicrobial activity against *Escherichia coli*, *Proteus vulgaris*, *Salmonella enteritidis*, *Bacillus subtilis*, *Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniaeS, Xanthomonascitri, Candida albicans, C. tropicalalis, C.glabreta, Aspergillus niger, A. fumigatus, A. flavus,* and *Fusariumoxysporum*. Therefore, using the agar-well diffusion method, the current study shall assess the in vitro antibacterial activity of *Euphorbia turicalli* extracts against *E. coli* responsible for causing colibacillosis in poultry flocks.

Figure 2: Euphorbia turicalli plant

2.2 Resistant strain for *E.coli* responsible for causing colibacillosis in poultry flocks.

Avian pathogenic *Escherichia coli* is a causative agent for colibacillosis in poultry flocks worldwide resulting in mortality affecting the poultry industry (Dziva & Stevens, 2008). It is a threat to poultry life and poultry environments. The poultry sector is looking for non-antibiotic substitutes to help treat illnesses caused by avian pathogenic *E. coli*. The use of third- or fourth-generation cephalosporins in food animals must end. This appears to be especially crucial for chickens where antibiotics are administered in large quantities through automated injections into the feed and water. Avian pathogenic *E. coli* strains are beginning to exhibit resistance to the

recognized antibiotics that are used to treat them (Saidi *et al.,* 2013). Farmers that raise poultry have resorted to medicating their sick birds using herbs. It is advisable that Zimbabwe adopt the same strategy as the broiler sector in the United States, which has moved the majority of its output to never using antibiotics. This can be achieved by research on the use of alternatives such as plant extracts to treat sick birds.

APEC affects birds of all ages and different types of poultry production including broiler and commercial layers and breeders. It poses a threat to the economy due to the high rates of morbidity and mortality. In Zimbabwe, we are facing a challenge with antibiotics because bacterial diseases are treated without first establishing an anti-bigram. Without these surveys to determine antibiotic resistance profiles the use of antibiotic to treat bacterial infections can be irrational (Gamberini *et al.,* 2023).The use of antibiotics without determining antibiotic resistance profile may cause untreatable, and prolonged bacterial infections in humans, leading to higher healthcare cost and even rise in number of deaths.

Escherichia coli is a gram negative facultative anaerobic bacilli that are part of the normal intestinal micro flora of poultry. Although most *E. coli* are non-pathogenic, some strains are able to establish themselves outside of the intestines and cause disease (Leimbach *et al.,* 2013). These strains are known as Avian Pathogenic *E. coli (APEC).* *APEC* is the major cause of colibacillosis in poultry production. Colibacillosis is commonly characterized by tread of lesions, perihepatitis, yolk sac infections, swollen head syndrome, pericarditis and airculitis resulting in septicemia and early death (Nolan *et al.,* 2013). The severity of avian pathogenic *E.coli* depends on health status of the host. Scientific studies have shown that the gas exchange site of the lung and the interstitium of the air sacs are the most important sites of entry of avian pathogenic *E.coli. (*Dho-Moulin & Fairbrotger, 1999). Avian Pathogenic *E.coli* is a zoonotic pathogen.

According to Abdelwahab *et al.* (2022), the development of multidrug-resistant *E. coli,* a hazard to public health, has been connected to the use of antimicrobials in the treatment of livestock or as a growth stimulant. It has been documented that *E.coli* isolated from livestock samples has varying resistances to many antimicrobials, such as gentamicin, ampicillin, tetracycline, erythromycin, sulfamethoxazole, chloramphenicol, kanamycin and streptomycin. Due to treatment failure costs and the extended duration of treatment required for bacterial infections, farmers are also negatively impacted financially by this resistance phenomena. According to a study carried out in Emirate of Abu Dhabi, UAE in the years 2014-2019, *E.coli* strains isolated from livestock showed multi drug resistance to a number of drugs commonly used in the treatment of livestock by farmers. Based on the results obtained in the study of *E.coli* strains from Abu Dhabi all isolates 165/165 (100%) proved to be phenotypically and genotypically multidrug-resistant carrying one or more resistant determinants (Abdelwahab *et al.,* 2022). The

most dominant antimicrobials that were found to have high resistance were ampicillin, tetracycline, and co-trimoxazole, and the lowest resistance was enrofloxacin.

In Malaysia, a study was carried out in certain Malay villages to ascertain the prevalence of *Escherichia coli* and *Campylobacter jejuni* as well as their profiles of antibiotic resistance in humans, chickens, wild birds, and the surrounding environment. In this investigation, 359 samples were gathered from humans (47), chickens (71), wild birds (38), and the environment (153). The *C. jejuni* and *E. coli* isolates were subjected to the disc diffusion method's antibiotic test. 89.4% of human samples, 47.4% of avian samples, 44.6% of chicken samples, and 71.2% of environmental samples tested positive for E. coli. The isolates' susceptibility to ten antibiotics was assessed. It was discovered that 100% of the E. coli isolates and 84% of the *C. jejuni* isolates were resistant to at least one antibiotic. Tetracycline and cefpodoxime resistance was high in the isolates (Mohamad *et al.*, 2022).

Additionally an investigation was carried out in Harare, Zimbabwe to establish the trends and prevalence of antibiotic resistance in Avian Pathogenic *E. coli*. 503 chickens with colibacillosis were diagnosed; 103 E. coli isolates were collected from them. The isolates' sensitivity to eight marketed antibiotics was assessed using the disc diffusion method. Multiple antibiotic resistance was present in many of the isolates. Maximum resistance to tetracycline (100%), bacitracin (100%), and cloxacillin (10%) was found in antibiogram profiles, while ampicillin resistance was quite prevalent (94.1%). Results also showed significant prevalence of gentamycin (97.1%) and ciprofloxacin (100%) sensitivity. The isolates' frequencies of neomycin and chloramphenicol sensitivity were moderate. Every sample in this showed resistance to three or more drugs, multidrug resistance was evident in every isolate. Seven patterns of multidrug resistance were found. According to the research carried out, resistance to drugs related to colibacillosis in Zimbabwe is on the rise in Avian Pathogenic *E.coli*, this significant amount of multidrug resistance may make it more difficult for poultry farmers to treat APEC in Zimbabwe (Saidi *et al.*, 2013).

2.3 Extraction solvents used for extracting phytochemical components

The form of solvent used for the extraction process has a significant impact on the outcome of the process of determining biologically active chemicals from plant material. Good plant extraction solvents have the following qualities: minimal toxicity, simple evaporation at low temperatures, quick physiologic extract absorption, preservation activity, and resistance to complexation or dissociation of the extract. A number of factors influence the selection of the solvent, including the amount of phytochemicals to be extracted, the rate of extraction, the variety of different compounds extracted, the diversity of inhibitory compounds extracted, the ease of handling the extracts in the future, the solvent's toxicity during the bioassay process, and any potential health risks associated

with the extracts (Jain *et al.,* 2015). The planned use of the extract influences the solvent selection. The solvent should not be harmful and not impede the bioassay because the final result will have residues of the solvent. The decision will also be influenced by the specific molecules that need to be removed (Prasad *et al.,* 2011).

2.3.1 Distilled water as an extraction solvent

Water is a ubiquitous solvent that is employed in the extraction of antibacterial plant compounds. While water is the primary healing medium used by traditional healers, research has shown that plant extracts derived from organic solvents have more stable antibacterial action. Moreover, water soluble phenolics are solely significant as an antioxidant compound, while water soluble flavonoids mostly anthocyanins have no antibacterial importance (Zeljkovic *et al.,* 2020). Hayat *et al.,* (2016) used water to assess the crude extract made from *Aloe vera* L. leaves' in vitro antibacterial activity. Ethanol and methanol were two of the solvents used to create the extract. The agar well diffusion method was used to assess the extract's inhibitory capability against a variety of bacteria such as *Staphylococcus aureus, Candida albicans, Salmonella typhi, Salmonella carotovora, Klebsiella pneumoniae, Bacillus subtilis,* and *Bacillus cereus*. The concentrations of the extracts were 30 µL and 60 µL. The three standards—Clotrimazole (50 µg mL–1) for *Candida albicans*, Ciprofloxacin (30 µg mL–1) for Gram-negative bacteria, and Azithromycin (50 µg mL–1) for Gram-positive bacteria—were used to measure and compare the zone of inhibition.

2.3.2 Acetone as a solvent

Acetone is an excellent extract, particularly in antimicrobial studies where more phenolic compounds need to be extracted. It dissolves many hydrophilic and lipophilic components from the two plants used, is volatile, miscible with water, and has a low toxicity to the bioassay used. According to a study, aqueous acetone extracts tannins and other phenolic more effectively than aqueous methanol. It was discovered that saponins with antibacterial action may be extracted using both methanol and acetone (Nurelhuda *et al.,* 2010).

2.3.3 Methanol as an extraction solvent

Many plant components can be extracted using methanol as the solvent. It has been successful in extracting a variety of plant components, such as alkaloids, flavonoids, phenolic compounds, and other bioactive substances. Plant materials can effectively provide polar and moderately polar chemicals by the use of methanol, a polar solvent. Because of its strong solubility for a wide range of phytochemicals, it can be used to thoroughly extract a

variety of plant components. Due to its low boiling point of 64.7°C and ease of evaporation under moderate pressure, methanol makes it possible to recover the extracted chemicals with less residue (Badawy *et al.*, 2022)

Methanol is a versatile and widely-used solvent due to its several key advantages. As a polar protic solvent, methanol can effectively dissolve a wide range of organic and inorganic compounds, making it useful in various chemical processes and applications. Additionally, its low boiling point and high vapor pressure allow for easy evaporation and recovery, making it suitable for use in paints, coatings, and inks. Moreover, methanol's miscibility with water and other organic solvents enables the formulation of tailored solvent mixtures to meet specific requirements (Badawy *et al.*, 2022).

CHAPTER 3

METHODS AND MATERIALS

3.1 Study Area

The research was carried out at the Department of Veterinary and Technical Services, Harare.

3.1 Experimental Design

Plates were divided into four quadrants per plate and used for testing the antibacterial activity of three different concentrations (25%, 50%,75%) of the crude extracts for *A. vera* and *Euphorbia turicalli* against the resistant *E. coli* bacterial strain respectively. Wells of 6 mm diameter were made using a cork borer, in each quadrant and a particular concentration (25mg/ml, 50mg/ml and 75mg/ml) of the crude extract to be tested was added. Methanol, acetone and distilled water were used as extraction solvents. Zones of inhibition for each extract against the bacterial strain were recorded after 24hours of incubation at 37 degrees celsius. Starting from the stock solution which was at 100% serial dilution were conducted for acetone, methanol and aqueous *Euphorbia turicalli extracts.* Serial dilutions of the extracts were made in liquid medium (Nutrient Broth) which was inoculated with a standardized bacterial suspension and incubated for a prescribed time of 24 hours. The lowest concentration (highest dilution) of the extract preventing appearance of turbidity was considered to be the minimal inhibitory concentration (MIC). The Minimum Inhibitory Concentrations were recorded. All the experiments were performed in triplicates.

Table 1: Showing the experimental design for the first experimental replicate

	Aloe vera	*Euphorbia turicalli*	+ve	-ve

	Aqueous extract	Methanolic extract	Acetone Extract	Aqueous extract	Methanolic Extract	Acetone extract	control	control
25%	A_1 A_2 A_3	A_1 A_2 A_3	A_1 A_2 A_3	E_1 E_2 E_3	E_1 E_2 E_3	E_1 E_2 E_3	C_1 C_2 C_3	C_1 C_2 C_3
50%	A_1 A_2 A_3	A_1 A_2 A_3	A_1 A_2 A_3	E_2 E_2 E_3	E_1 E_2 E_3	E_1 E_2 E_3		
75%	A_1 A_2 A_3	A_1 A_2 A_3	A_1 A_2 A_3	E_1 E_2 E_3	E_1 E_2 E_3	E_1 E_2 E_3		

Key:

$A_{:}$ = *Aloe Vera* replicates

E : *Euphorbia turicalli* extract

C: Controls

Subscript 1-3: represents the plate replicates

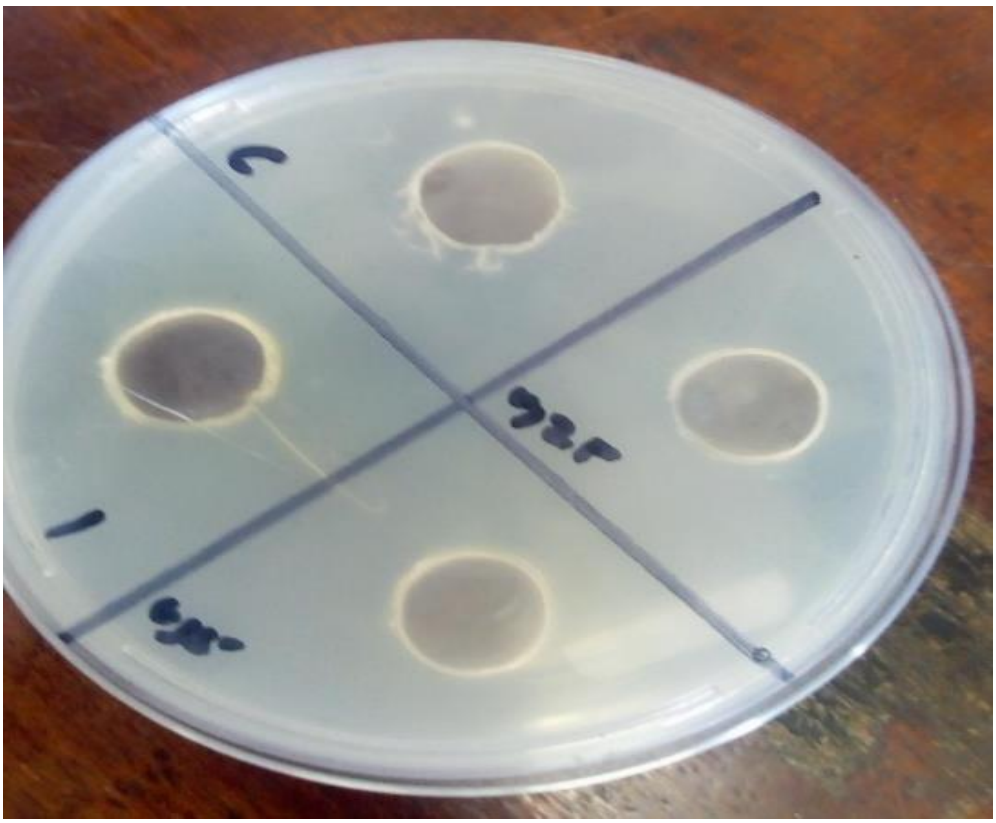

Figure 3: A typical plate used for determining Inhibition zones of different concentrations

3.3 Plant Sample Collection

Fresh plant leaves of *E. turicalli* and *A. vera* were collected from the Botanic Garden in Harare, Zimbabwe. The voucher specimens were transported at room temperature and were deposited at the Toxicology section at Central Veterinary Laboratory. The samples were wrapped to keep them away from sunlight to avoid wilting. Species identification was carried out by a qualified botanist. Before use samples, they were washed using distilled water.

3.4 Bacterial strains

The antibacterial activity of each extracts, of *Euphorbia turicalli* and *A.vera* were evaluated using a strain of avian pathogenic *E.coli* ST141. The bacterial strains used were from the culture collection of the Bacteriology section.

3.5 Media preparation

All the media prepared were sterilized by autoclaving the media at (121°C). Pure cultures of all experimental bacteria were obtained from Department of Veterinary and Technical services , Bacteriology Section. Media was prepared as per manufacturer's guide.

3.6 Inoculum preparation

The bacterial cultures were resuscitated by culturing on blood and MacConkey agar at 37^{o}C for 16 hours. Using a sterile cotton swab, top of 4-5 single colonies were transferred to a tube of 0.85% saline. The inoculums standard were adjusted to a 0.5McFarland and the turbidity was compared to that in the 0.5 McFarland standard using a paper with black lines. After 15 minutes of preparing the inoculums, a sterile cotton swab was dipped, and streaked the swab over the enter surface of Mueller- Hinton agar, rotating the plate approximately 60^{o} then repeating the streaking motion. The plate was rotated 60^{o} again and streaking was repeated for the third time. Complete inoculation by running the swab around rim of agar. The lid was left ajar to allow for any excess moisture to be absorbed before punching the holes on the inoculated plates.

3.7 Extraction Method

Whole aerial fresh plant part of *Euphorbia turicalli* and leaves of *Aloe vera* were separately dried in an oven at 50 ^{o}C for forty eight hours (Marume *et al.*, 2017). *E. turicalli* leaves, and *A. vera* leaves were weighed using a balance

scale. Plant materials were ground in a homogenizer and extracted with methanol, acetone and distilled water. *E. turicalli* was measured and added into 100ml of methanol, 100ml of acetone and 100ml of distilled water respectively. The procedure was repeated for A. *Vera.* The extracts were stored at *5^{o}C.* All the 50% methanol extracts and aqueous extracts were filtered using Whatman's filter paper. Stock solutions were prepared thus for Euphorbia *turicalli* and *A. vera* for ethanol, acetone and aqueous extracts respectively. Concentrations of 75mg/ml,50mg/ml and 25mg/ml of the two different plant extracts stock solutions were prepared and stored at 4^{o}C.

3.8 Preparation of dilutions

Three universal bottles were labelled 25mg/ml, 50mg/ml and 75mg/ml for each of the methanol, acetone and aqueous filtered extracts respectively. Distilled water was used as the negative control. Commercially bought antibiotic was used as a positive control.

3.9 Screening for the antibacterial activity of extracts

The Agar well diffusion method was used to evaluate the antibacterial activity of each of the crude extracts (Zaidan et al., 2005). Each of the plant extract were dissolved in 100ml of methanol and distilled water respectively. Mueller-Hinton agar was poured into each plate and was left to set. The wells were made using a 6mm diameter borer. The extracts were loaded in each well respectively. Distilled water was added in the control wells as negative control. The presence of inhibition zones were measured using a vanier caliper and this was considered as indication for antibacterial activity. The disk well diffusion method was also used to determine the inhibition zones of filtered plant extracts. The procedure was performed in triplicates.

3.10 Determination of MICs of plants extracts

For determination of the MICs of the plant extract the procedure adopter by Andrews, (2001) was adopted. Filtered plant extracts of a concentration of 25mg/ml that showed inhibition zones using the agar well diffusion method were then tested to determine their Minimum Inhibitory Concentrations. Serial dilutions of the extracts were made in liquid medium (Nutrient Broth) which was inoculated with a standardized bacterial suspension and incubated for a prescribed time of 24 hours. The lowest concentration (highest dilution) of the extract preventing appearance of turbidity was considered to be the minimal inhibitory concentration (MIC).Sterile capped test tubes were grouped and numbered from 1 to 7. All of the steps were carried out using aseptic techniques. 2.0 ml of the filtered plant extract (25mg/ml) was added to the first tube. 1.0 ml of sterile nutrient broth was added to all the tubes. 1ml from the first tube was added to the second tube. Using a separate pipette, the contents of the second tube were mixed and 1.0 ml was transferred to the third tube. The dilutions were continued in this manner to test tube number 5, precaution being taken to change pipettes between tubes to prevent carryover of extract on the external surface

of the pipette.1.0 ml was removed from tube 5 and was discarded. An overnight bacterial suspension was added into 40.0ml of sterile nutrient broth to give a slightly turbid suspension. 1ml of each diluted bacterial suspension was added to each of the tubes giving the final concentration of each test tube being half of the original concentrations. The test tubes were incubated at 37^{o} C for 24hours. The tubes were examined for visible signs of bacterial growth. The highest dilution without growth was considered to be the minimal inhibitory concentration (MIC).

3.11 Data analysis

One way ANOVA was used to assess the mean difference for the inhibition zones for *Aloe vera* and *Euphorbia turicalli.* Results were presented using descriptive statistics in form of bar graphs and tables.

CHAPTER 4

RESULTS

4.1 The antibacterial activity of extracts of *Euphorbia turicalli* extracts against avian pathogenic *Escherichia coli* (APEC)

A gradual increase in the diameter of the Zone of inhibition after treatment with the Methanol extract was noted indicating that the methanol extract acted as an antimicrobial against the *E.coli* strain. Extracts for *Euphorbia turicalli* reflected antibacterial inhibitory properties against *E. coli*. The methanol extract at a concentration of 75% had the largest inhibition zones against the avian *E. coli* strain with a mean diameter of 7±0.00mm. A gradual increase in the diameter of the Zone of inhibition after treatment with the acetone extract was noted. The largest mean zone of inhibition for the acetone extract against *E.coli* was 3.7±0.14 mm. The zone of inhibitions noted after treatment with different concentration of the water extract were relatively small compared to those of methanol and acetone extract. The largest zone of inhibition for *Euphorbia turicalli* aqueous extract identified was 2.2±0.00mm.

Table 2: Inhibition Zones for *Euphorbia turicalli* extracts against avian pathogenic *E. coli*

Bacteria species	**Diameter of Inhibition Zones(mm) ± SD**								
	Methanol Extract			*Acetone Extract*			*Aqueous Extract*		
	25%	50%	75%	25%	50%	75%	25%	50%	75%
E.coli	3.7 ± 0.14	1.7 ± 0.71	7.0 ± 0.00	2.3 ±0.13	3.4 ±0.00	3.7 ± 0.23	0.7 ± 025	1.2 ±0.00	2.2 ± 0.00

4.2 The antibacterial activity of extracts of *Aloe vera* extracts against avian pathogenic *Escherichia coli* (APEC)

A gradual increase in the diameter of the Zone of inhibition after treatment with the *Aloe vera* Methanol extract was noted indicating that the methanol extract acted as an antimicrobial against the *E.coli* strain. The methanol extract at a concentration of 75% had the largest inhibition zones against the avian *E. coli* strain with a mean diameter of 9.5± mm. A gradual increase of the diameter of the Zones of inhibition after treatment with the *Aloe vera* acetone extract was noted. The largest mean zone of inhibition for the acetone extract against *E.coli* was 7.7 ± 0.23mm. The zone of inhibitions noted after treatment with different concentration of the water extract were relatively small compared to those of methanol and acetone extracts. The largest inhibition zone for aqueous extract was 4.6±0.0mm.

Table 3: Inhibition Zones for *Aloe vera* extracts against avian pathogenic *E. coli*

Bacteria species	**Diameter of Inhibition Zones(mm) ± SD**								
	Methanol Extract			*Acetone Extract*			*Aqueous Extract*		
	25%	50%	75%	25%	50%	75%	25*	50%	75%
E.coli	4.7 ±0.14	5.7 ±0.71	9.5 ±0.00	4.3 ±0.13	6.4 ±0.00	7.7 ±0.23	2.4 ±025	4.2 ±0.00	4.6 ±0.00

4.3 The antibacterial effects of *Euphorbia turicalli* and *Aloe vera* extracts against avian pathogenic *Escherichia coli* (APEC)

A p value of 0.03 indicates a significant difference in the antibacterial effects of Euphorbia *turicalli* and *Aloe vera* extracts against avian pathogenic *Escherichia coli* (APEC)

Table 4. Two inhibition zones for *Euphorbia turicalli* and *Aloe vera* extracts against avian pathogenic *Escherichia coli* (APEC)

Tests of Between-Subjects Effects					
Dependent Variable: Aloe_vera					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	.704[a]	1	.704	.628	.032
Intercept	296.704	1	296.704	264.836	.000
Euphorbia_turicalli	.704	1	.704	.628	.032
Error	53.776	48	1.120		
Total	362.000	50			
Corrected Total	54.480	49			
a. R Squared = .013 (Adjusted R Squared = -.008)					

4.4 The minimum Inhibitory concentrations for *Euphorbia turicalli* and *Aloe vera* against *E. coli*.

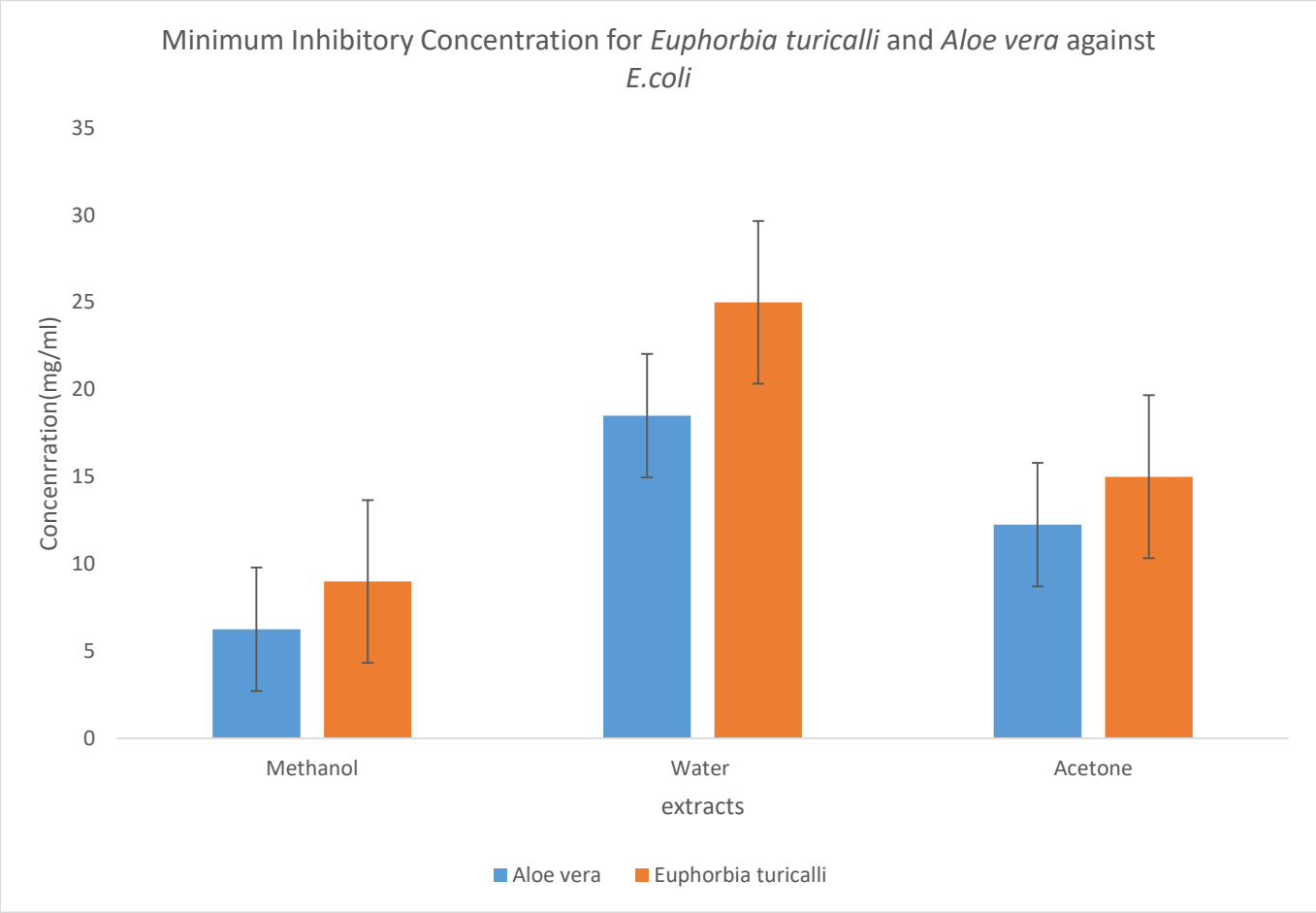

Figure 4. The minimum inhibitory concentration for *Euphorbia turicalli* and *Aloe vera* against *E. coli*

The Minimum Inhibitory Concentration for *Euphorbia turicalli* and *Aloe vera* against *E. coli* ranged from 6.25mg/ml to 25mg/ml. The most effective extract was the methanol *Aloe vera* extract with an MIC of 6.25mg/ml. For both methanol, water and acetone extracts *Aloe vera* extracts were relatively effective with MIC values of 6.25mg/ml, 18.5mg/ml and 12.25mg/ml respectively. The minimum inhibitory concentrations for *Aloe vera* were in water, methanol and acetone were statistically significant, (0.03). The minimum inhibitory concentrations for *Euphorbia turicalli* were in water, methanol and acetone were statistically significant, (0.04).

CHAPTER 5

DISCUSSION

5.1 The antibacterial activity of extracts of *Euphorbia turicalli* extracts against avian pathogenic *Escherichia coli* (APEC)

All *Euphorbia turicalli* extracts reflected antibacterial inhibitory properties. The methanol extract at a concentration of 75% had the largest inhibition zones against the avian *E. coli* strain with a mean diameter of 7mm. The findings are similar with the results of Ekhuemelo *et al.*, (2021). The study asserts that *Euphorbia turicalli* is an effective antifungal and antimicrobial agent. The inhibition zones for their study were relatively larger than those of the present study. The difference in these results might be because of the different extraction methods used in the two study. This is supported by Wal *et al.*, (2013) who reports that the antimicrobial activity of *Euphorbia turicalli* is influenced by the extraction method used. A gradual increase in the diameter of the Zone of inhibition after treatment with the acetone extract was noted. The largest mean zone of inhibition for the acetone extract against *E.coli* was 3.7 mm. The zone of inhibitions noted after treatment with different concentration of the water extract were relatively small compared to those of methanol and acetone extract. The largest zone of inhibition for *Euphorbia turicalli* aqueous extract identified was 2.2mm. ANOVA was performed to assess the inhibition zone mean differences for the different extract and a p value of 0.01 was obtained indicating a significant differences in the means. The results of this study, however, are not as high as those reported by Waheed *et al.* (2020), who found that at concentrations of 500 mg mL-1 and 1,000 mg/mL, respectively, the zones of inhibition were 24 and 32 mm on Rhizopus nigricans, 16 and 34 mm on Acremonium, and 28 and 34 mm against *A. niger* from an aqueous *Euphorbia helioscopia* extract. According to Guevara, (2005), values of the antibiotic's zone of inhibition less than 10 mm are considered inactive, values between 1 and 4 mm are considered partly active, values between 5 and 19 mm are considered active, and values beyond 19 mm are considered extremely active. This

suggests that the *E. tirucalli* extracts described from this investigation were highly effective in controlling *E. coli* prevalence. The result are useful in surveillance of new therapeutics remedies for resistant avian pathogenic *E.coli.*

5.2 The antibacterial activity of extracts of *Aloe vera* extracts against avian pathogenic *Escherichia coli* (APEC)

The methanol extract at a concentration of 75% had the largest inhibition zones against the avian *E. coli* strain with a mean diameter of 9.5mm. A gradual increase of the diameter of the Zones of inhibition after treatment with the *Aloe vera* acetone extract was noted. The largest mean zone of inhibition for the acetone extract against *E.coli* was 7.7 mm. The zone of inhibitions noted after treatment with different concentration of the water extract were relatively small compared to those of methanol and acetone extracts. The largest inhibition zone for aqueous extract was 4.6mm at a concentration of 4.6. ANOVA was performed to assess the inhibition zone mean differences for the different extract and a p value of 0.02 was obtained indicating a significant differences in the means. The findings resonate with the results of Mahdi *et al.,* (2017) in a study that assessed the antibacterial properties of *Aloe vera* aqueous extract against a clinical isolate of *E. coli.* The Minimum Inhibitory Concentration for *Euphorbia turicalli* and *Aloe vera* against *E. coli* in the present study ranged from 6.25mg/ml to 25mg/ml. The most effective extract was the methanol *Aloe vera* extract within MIC of 6.25mg/ml. For both methanol, water and acetone extracts Aloe vera extracts were the most effective with MIC values of 6.25mg/ml, 18.5mg/ml and 12.25mg/ml respectively. In the study of Mahdi *et al,* (2017) the *Aloe vera* extract's minimum inhibitory concentration (MIC) was 2.23 mg/mL, and it proved effective in preventing *E. coli* from growing.

CHAPTER 6

6.1 Conclusion

It has been demonstrated that both extracts from *E. tirucalli* had antibacterial properties against E. coli. Zones of inhibition and MIC values found for *E. tirucalli* extracts suggest that the species was highly effective and might be applied to the management of illnesses brought on by the avian pathogenic *E. coli.*

6.2 Recommendations

The study also recommends the use of different extraction methods of the phytochemical components for *E. tirucalli* to enhance the efficacy of *Euphorbia turicalli* and *Aloe vera* .The study also recommends further on the efficacy of *Euphorbia turicalli* and *Aloe vera* on other resistant strains like MRSA.

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APPENDIX

The antibacterial activity of extracts of *Euphorbia turicalli* extracts against avian pathogenic *Escherichia coli (APEC)*

Methanol extract

ANOVA

Methanol_extract

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.144	2	1.072	2.074	.029
Within Groups	76.491	148	.517		
Total	78.636	150			

Ethanol extract

ANOVA

Ethanol_extract

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.221	2	.111	.473	.024
Within Groups	34.339	147	.234		
Total	34.560	149			

Aqueous extract

ANOVA

Aqueous_extract

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.346	2	.173	.679	.010
Within Groups	19.654	77	.255		

Total	20.000	79			

The antibacterial activity of extracts of Aloe vera extracts against avian pathogenic Escherichia coli (APEC)

Methanol extract

ANOVA

Methanol_extract

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.549	2	.774	3.674	.028
Within Groups	31.193	148	.211		
Total	32.742	150			

Ethanol extract

ANOVA

Ethanol_extract

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.804	7	.115	.577	.001
Within Groups	9.758	49	.199		
Total	10.561	56			

Aqueous extract

ANOVA

Aqueous_extract

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.243	7	1.320	3.844	.007
Within Groups	7.557	22	.344		
Total	16.800	29			

ANOVA analysis for *Aloe vera extracts*

ANOVA - Single Factor

Alpha 0.05

Groups	Count	Sum	Mean	Variance
Column 1	3	32	10.66666667	1.333333333
Column 2	3	21	7	4
Column 3	3	15	5	1

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	49.55555556	2	24.77777778	11.73684211	0.00843627	5.14325285
Within Groups	12.66666667	6	2.111111111			
Total	62.22222222	8				

ANOVA analysis for *Euphorbia turicalli extracts*

ANOVA - Single Factor

Alpha 0.05

Groups	Count	Sum	Mean	Variance
Column 1	3	18	6	0
Column 2	3	16	5.333333333	0.333333333
Column 3	3	7	2.333333333	1.333333333

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	22.88888889	2	11.44444444	20.6	0.002054129	5.14325285
Within Groups	3.333333333	6	0.555555556			
Total	26.22222222	8				

ANOVA - Single Factor

Alpha	0.05

Groups	Count	Sum	Mean	Variance
Column 1	3	37	12.33333333	0.333333333
Column 2	3	29	9.666666667	0.333333333
Column 3	3	18	6	1

Source of Variation	SS	df	MS	F	P-value	F critical
Between Groups	60.66666667	2	30.33333333	54.6	0.000141285	5.14325285
Within Groups	3.333333333	6	0.555555556			
Total	64	8				