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DEPARTMENT OF BIOLOGICAL SCIENCES

Comparative Analysis Of *Syzygium Aromaticum* And *Punica Granutum* Controlling Coccidiosis In *Gallus Domesticus.*

By

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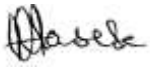
APPROVAL FORM

Title of dissertation: A comparative analysis of *Syzygium aromaticum* and *Punica granatum* in controlling coccidiosis in *Gallus domesticus*.

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

Signature of student  Date**……..……28/09/2024**

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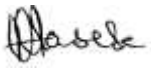
Signature  Date…….28/09/2024.

DEDICATION

I dedicate this project to my parents Prince and Iylene Masebe for being by my side throughout the academic journey.

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
PCR	Polymerase Chain Reaction
SPSS	Statistical Package for the Social Sciences

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ABSTRACT

Coccidiosis has become a major threat in the poultry industry causing high mortality rate in poultry. The protozoan disease has reduced the economic stability due to drug resistance thus demanding more attention. The study aimed to evaluatig the effect of *Syzygium aromaticum* and *Punica granutum* in controlling coccidiosis in ross broilers. Methanolic, ethanolic and aqueous extraction were used for each plant. A total of twenty five, 21 days old chicks were devided into 4 groups and 20 chickens were infected. The infected groups were treated with *S.aromaticum, P. granutum* and Ampolium. Ampolium is a commercial drug which work as an coccidiostat (Gerhold, 2023). Parameters such as oocyst reduction and weight gain were recorded at day 7, day 14 and day 28. The fecal oocyst count was done using the McMaster technique. All data collected was subjected to analysis of variance (ANOVA) at $\alpha = 0.05$ level of significance. Faecal oocyte shedding decreased significantly ($p < 0.05$) in the treatment groups that were treated with *S.aromaticum* and *P.granatum*. The mean weight of what? deviation from day 7 to day 14 was great for the methanol extract of the herbal extract of *Syzygium aromaticum*(540g), followed by methanolic extracts for *Punica granatum* (430g),and finally ethanolic extracts of *Syzygium aromaticum* (320g) and ethanolic extracts of *Punica granatum*(300g). The trending model for *S. aromaticum* in terms of weight deviation ($y = 10x+380$) showed that it has a greater efficacy against coccidiosis. Methanolic extracts treated groups exhibited better improvement, followed by ethanolic and finally distilled water in terms of oocyst reduction. The lesion score for untreated group was 3, methanolic extraction for *S.aromaticum* 1, *P.granatum* was 2 and for the ethanolic extracts for both herbs the lesion score was 2 which showed that *S.aromaticum* exhibited better efficacy in the treatment of coccidiosis. The study provided a baseline assessment for the treatment of coccidiosis . The significance of *Syzygium aromaticum* and *Punica granatum* in eradiaction of coccidian have been recognised as a possibility upon proper solvent extraction and observation of the minimum dose required to counteract the effects of the disease.

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CHAPTER 1

1.1 INTRODUCTION

Coccidiosis is the most common disease affecting poultry. It is caused by an internal parasitic protozoan of the genus *Eimeria*, which leads to significant financial losses for the poultry industry. This illness spreads through the fecal-oral route and is endemic in tropical and subtropical regions. Coccidiosis is a prevalent intestinal disease that affects domestic chickens (Gallus gallus domesticus) in particular, resulting in major economic losses. The causative agents are several *Eimeria* species, including E. tenella, E. maxima, E. mitis, E. praecox, E. hagani, E. brunette, E. acervulina, E. necatrix, and E. mivati (Conway & McKenzie, 2008). These can lead to intestinal ulcers in infected birds (Gadzirai, 2005). Coccidiosis occurs by the ingestion of sporulated oocyst.Clinical signs include diarrhea with bloody droppings, weight loss, lethargy, loss of appetite, ruffled feathers, pale comb, droopiness and depression (Abdel, 2019). Plants have been the primary source of medicines for centuries, particularly as resistance to antiprotozoal drugs has increased over time. Traditionally, crude extracts derived from various plant parts like roots, stems, bark, flowers, fruits, and seeds have been widely used to treat a variety of diseases, including parasitic infections. However, the treatment and control of coccidiosis in poultry is predominantly achieved through the use of pharmaceutical drugs and vaccines.

Resistance refers to the ability of a parasite or pathogen to survive treatment with an antiparasitic or anticoccidial drug that was previously effective against it. *Eimeria* infections in poultry can disrupt digestion and nutrient absorption in the gastrointestinal tract, leading to immunosuppression and reduced productivity. These infections can also increase the number of poultry carcasses rejected at the slaughterhouse (Karimi, 2021). Since plant-derived products are currently being explored and have shown promise in controlling protozoan diseases without the use of antibiotics, this suggests a lower-cost and potentially more effective approach compared to chemical compounds. This alternative approach would likely be more welcomed by the poultry industry.

According to studies and research, it has been found that pomegranate peel extract and cloves have promising antiparasitic properties against *Eimeria*, the causative agent of coccidiosis in poultry. This particular study evaluated the effects of cloves and pomegranate peel extract on *Eimeria* parasites, using indicators such as reduced oocyst (egg) excretion, improved weight gain, and decreased severity of pathological intestinal lesions in broiler chickens (Mufarrej,2019).

1.2 PROBLEM STATEMENT

In all parts of the world where confinement rearing is practised, coccidiosis represents a major disease problem demanding the attention of poultry producers, feed manufactures, and poultry disease experts. Avian coccidiosis is a growing threat to poultry industry causing economic losses in production (Abbas, 2012). Anticoccidial resistance is a threat for commercial farmers because it results in the wastage of money and resources for controlling the disease. Anticoccidial drugs are becoming less effective in treating coccidiosis due to the increasing resistant strains of *Eimeria.* Antiparasitic resistance occurs

when parasites mutate and develop the ability to resist anticoccidants and antiparasitic drugs (Blake 2011). Although the strategy of using preventive chemical compounds has been very effective in controlling parasitic infections, the emergence of drug resistance and chemical residues in animal and poultry tissues has caused great concern and a threat to the consumer (Bahrami et al., 2014) and researchers have been looking for healthy alternatives such as use of herbal extracts (Pop et al., 2019).

1.3 SIGNIFICANCE OF STUDY

Antiprotozoan resistance has become a global concern especially in developing countries.Plant based therapy is now increasingly being adopted by low and middle income countries including Zimbabwe. Much of the ongoing research is being conducted by leading pharmaceutical companies in developed countries and recent studies have shown a great potential for plant based therapy for the treatment of coccidiosis (Naheda, 2022). The findings of this study will contribute to the development of alternative strategies for the control of coccidiosis in poultry. If proven effective, *Syzygium aromaticum* and *Punica granatum* extracts may serve as natural and sustainable alternatives to traditional chemotherapeutic agents. The results will have implications for the poultry industry, promoting the use of natural products for disease control and reducing reliance on synthetic drugs.

Other public health issues related to anticoccidial drugs are that the anticoccidial drug metabolites escape the circulatory system and deposited in different body parts. Secondary metabolites are transferred to consumers when they consume meat from animals that have been treated with anticoccidial drugs and these drug residues causes problems and can be carcinogenic. In most developed countries especially countries in the European Union, they have been banned the regular use of the anticoccidial drugs and therefore for them to get the coccidiostats they have to have a prescription from the veterinary doctor. By this scientists are trying and thriving on producing herbal medicine for animlals to limit the use of coccidiostats that can cause harm to poultry and consumers of the products of poultry.

In the control program of coccidiosis, phytogenic compounds can be considered as a suitable alternative to chemical compounds. The phytogenic anti-coccidial compound, further inhibitory effects against *Eimeria* species can have low side-effects on important organs of poulty (Dehghani-Ashkezari et al., 2019).

1.4 AIM OF THE STUDY

The aim of this study was to investigate and evaluate the efficacy of two natural products, *Syzygium aromaticum* (clove) *and Punica granatum* (pomegranate), in the control of coccidiosis in *Gallus gallus domesticus.*

1.5 OBJECTIVES OF THE STUDY

The objectives of the study were to

- ★ To establish effectiveness of *Syzygium aromaticum* extract in reducing *Eimeria* oocytes count in broiler chickens.
- ★ . To establish the effectiveness of *Punica granatum* extracts in reducing *Eimeria* oocytes count in broiler chickens.
- ★ To compare the effectiveness of *P.granatum* and *S.aromaticum* against *Eimeria* oocyst.

1.6 RESEARCH QUESTIONS.

- ★ Does *Syzygium aromaticum* reduce *Eimeria* oocytes count in broiler chickens?
- ★ Does *Punica granatum* reduce *Eimeria* oocytes count in broiler chickens?
- ★ Which plant species, *S. aromaticum* or *P. granatum* is most effective in reducing Eimera oocytes count?
- ★ Which of the two plant species *S. aromaticum* or *P. granatum* has a greater antiparasitic activity against *Eimeria* oocytes*?*

1.7 HYPOTHESIS

H_o :There is no significant difference in the suppression and control of *Eimeria parasites* against *Punica granutum* and *syzigium aromaticum.*
H_1 : There is significant difference in the suppression and control of *Eimeria* against *Punica granutum* and *Syzygium aromaticum.*

1.8 LIMITATIONS OF THE STUDY

One major limitation of the study was through identification and characterization of the diseases using molecular diagnostic assay techniques due to lack of reagents and equipment .

1.9 DEFINITION OF TERMS

Coccidiosis

- ★ It is a parasitic infection caused by the protozoan parasite coccidia.

Eimeria

- ★ *Eimeria* is a genus of protozoan parasites that belong to the phylum Apicomplexa. These parasites are responsible for causing coccidiosis in various animals, including poultry.

Pomegranate

- ★ Pomegranate is a fruit-bearing deciduous shrub or small tree known scientifically as *Punica granatum.*

Clove

Clove is an aromatic spice derived from the flower buds of the clove tree (*Syzygium aromaticum).*

- ★ Avian

Avian refers to anything related to birds. In the context of avian coccidiosis, it pertains to the disease affecting birds, particularly poultry, caused by *Eimeria* parasites.

CHAPTER TWO

LITERATURE REVIEW

2.1 LIFE CYCLE OF *EIMERIA* SPECIES

Coccidiosis is a common parasitic disease caused by protozoan parasites belonging to the Apicomplexa phylum and Eimeriidae family (Muller, 2013). This disease affects a wide range of domestic and wild animals, including poultry, cattle, sheep, goats, and pigs. The economic impact of coccidiosis is significant, as it can lead to reduced productivity, increased morbidity, and high mortality rates in affected animals (Gerhold, 2023). Coccidiosis is a global issue, affecting various animal species across different geographical regions. The prevalence and severity of the disease depend on factors such as the host species, the specific coccidian species, environmental conditions, and management practices (Blake, 2010). The life cycle of the causative Eimeria parasites involves two stages: the exogenous phase, also known as sporogony, and the endogenous phase, which includes schizogony and gametogony (Cervantes, 2020). The sporozoite is the crucial stage that begins and completes the lifecycle. Sporozoites are the infectious form found within mature oocysts. They arise from the segmentation of the protoplasm within the oocyst. The protoplasm, known as the sporont, is surrounded by a resistant oocyst wall and is expelled from the host in the feces. After the external, environmental phase, the sporulated oocysts can initiate replication when ingested orally by a susceptible host, such as a chick. Inside the chick's intestines, the digestive enzymes and physical disruption cause the sporozoites to be released from the oocyst (Nabian,2018). Two key stimuli are required for the sporozoite to emerge that is carbon dioxide (CO_2) stress, which ruptures the micropyle and increases the permeability of the oocyst and the collapse of the oocyst contents due to the hypertonic salt solution in the intestine.The optimal concentration of CO_2 and incubation time required to trigger sporozoite release varies depending on the parasite species. Additionally compounds like bile and trypsin activate the sporozoites within the sporocyst and digest the Stieda body creating an opening in the sporocyst membrane. Bile facilitates the entry of digestive enzymes through the altered micropyle into the oocyst or it can modify the lipoproteins of the Stieda body in *Eimeria* oocysts. Trypsin digests the sporocyst wall along with parasite specific enzymes secreted by the activated sporozoites. Due to the continuous movement of the sporozoites the Stieda body of *Eimeria* species begins to swell and then disappears, leaving a small hole through which the sporozoites escape.

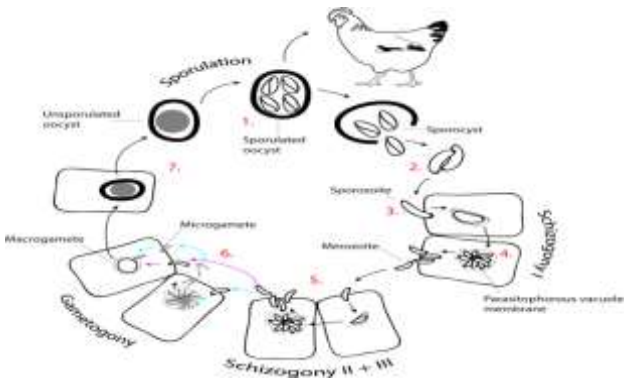
During the excystation and invasion of the host cell, the sporozoite utilizes its stored amylopectin as an energy source.The free sporozoites infect intestinal cells of the host gut and develop within a parasitophorous vacuole into a rounded, growing organism called the trophozoite. The trophozoite then becomes a meront during the first merogony generation.As the meront develops, the endothelial host cell becomes hypertrophic with an enlarged nucleus and nucleolus and its chromatin becomes scattered.

The host cell's cytoplasm organizes into two concentric zones and its nucleus drifts to the periphery to assist in meront development. Merogony begins with multiple nuclear divisions of the *Eimeria* trophozoite without cytoplasmic division resulting in the formation of ellipsoidal blastophores with a peripheral layer of nuclei. Merozoites then form around each nucleus and grow radially (Burrell, 2020). Finally cytoplasmic division produces the mononuclear, spindle-shaped, motile daughter cells called merozoites, which are separated by a residual body (Osorio, 2020).When the meront matures the merozoites rupture the host cell and escape into the intestinal lumen where they are carried to the large intestine. There, the merozoites 1 enter new epithelial cells and develop into second-stage meronts releasing second-stage merozoites (merozoites 2).After the second merozoites mature they attack adjacent cells and undergo sexual gamogony with most merozoites developing into a single, large, mononuclear, spheroid cell which is the female macrogamete.The macrogametes have eosinophilic granules which form an outer granule layer containing glycoproteins and an inner granule layer containing protein-rich molecules known as wall-forming bodies. Some merozoites 2 develop into large and polynucleated cells which then form many spindle-shaped cells with two flagella.

The gamonts quickly generate changes in the host cell causing it to lose its columnar structure. The pathological symptoms and clinical signs associated with *Eimeria* are characterized by the gamonts, as they cause degradation of the mucous membrane of the jejunum, ileum and cecum resulting in imbalances in absorption causing diarrhea (Deplazes, 2016).The free-released microgametes then fertilize the surrounding macrogametes forming zygotes. The eosinophilic granules in the macrogametes meet and form a resistant oocyst wall surrounding the zygote, which decreases in size and becomes a sporont. The *Eimeria* oocysts are finally released from the ruptured intestinal epithelial cells and excreted with the host's feces into the environment (Chartier et al.,2012). The un-sporulated *Eimeria* oocyst excreted from the host contains a diploid sporont stage which develops further through meiosis.The meiosis of the sporont generates four haploid sporoblasts which become enclosed by a shell forming sporocysts. Two sporozoites are then newly formed within each sporocyst. The sporont also generates a refractile polar body after meiosis. This exogenous sporulation process requires optimal environmental conditions including sufficient oxygen, moisture and adequate temperature (16%, 23°C) (Waldenstedt, 2001). Once sporulation is complete the metabolism and respiration of the oocyst are reduced. The oocyst uses its reserves of polysaccharides and eventually becomes non-infective as the parasite runs out of energy to carry out the final endogenous excystation process in the gut lumen. Sporulated oocysts may survive for long periods outside the host depending on environmental factors and temperature.

Figure 1 The life cycle of Eimeria

Fig 1 *Eimeria* shows life



cycle (Burrell et al.,2019)

2.2 Avian coccidiosis

The life cycle of coccidian parasites involves a series of distinct stages. This includes the initial sporulation process, where unsporulated oocysts develop into the infective sporulated form. The pathogenesis of coccidiosis the disease caused by these parasites is characterized by the invasion and infection of the host's intestinal epithelial cells by the merozoites. This invasion leads to significant tissue damage and inflammation within the intestine. The clinical signs associated with coccidiosis can vary widely depending on factors such as the specific coccidian species involved, the severity of the infection and the host species affected. The tissue damage and disruption of normal intestinal function caused by the parasite's lifecycle are central to the clinical manifestations of coccidiosis.The common clinical signs associated with coccidiosis include diarrhea, weight loss, anemia and dehydration in affected animals. In severe cases the disease can even progress to the point of causing death in the host. The diagnosis of coccidiosis is typically based on a combination of different methods. This includes the identification of the characteristic oocysts of the coccidian parasites within fecal samples from the affected animals. Histopathological examination of tissue samples can also provide evidence of the infection and associated tissue damage. Additionally more advanced molecular techniques such as polymerase chain reaction (PCR) assays can be utilized to detect and identify the specific coccidian species involved based on their unique genetic signatures. The use of these various diagnostic approaches from microscopic identification of oocysts to specialized molecular testing which helps to confirm the presence of coccidiosis and determine the causal coccidian species which is important for guiding appropriate treatment and control strategies.

Fig 2.2 Image of a broiler with coccidiosis (Roberts, 2009).

*Figure 2 Image of a broiler infected with coccidiosis (*Roberts, 2009).

2.3 Management, Prevention and Treatment of coccidiosis

The primary treatment approach for coccidiosis involves the use of various antiprotozoal drugs such as ionophores, diamidines and coccidiostats. The specific drug chosen depends on factors like the affected host species, the severity of the infection and the potential for drug resistance development. In addition to drug treatments, vaccination is also used as a preventative measure against coccidiosis particularly in the poultry industry. Vaccines containing live, sporulated coccidial species are administered to day old chicks at hatcheries. This allows the young birds to develop a protective T-cell mediated immunity against the coccidial strains included in the vaccine. The modern anticoccidial vaccines are designed to be given to the chicks on the day of hatch providing them with early resistance to the targeted coccidial species. This is important as the damage caused by coccidiosis can occur before clinical signs and symptoms become apparent.

Some anticoccidial drugs are also incorporated directly into the feed of animals, providing a prophylactic effect and helping to prevent the development of the disease. This prophylactic use of medications is considered the best approach as it can help mitigate the tissue damage and production losses associated with clinical coccidiosis. When administering therapeutic medications for the treatment of coccidiosis it is common to deliver them through the animals' drinking water rather than incorporating them into their feed. This is often a more practical and an effective approach given the logistical challenges of ensuring consistent medication intake through the feed. However beyond just the use of drugs the most important factor in preventing coccidiosis outbreaks is maintaining good sanitation practices. This includes keeping the housing and bedding for the animals clean and properly disinfected on a regular basis. Regularly removing and properly disposing of manure is also crucial as the oocysts of the coccidial parasites can contaminate the environment and spread the infection.

Proper ventilation within the animal housing is another key preventative measure. Adequate airflow helps to reduce moisture and ammonia levels both of which can contribute to the proliferation and spread of coccidiosis. Keeping feed and water sources away from areas where manure accumulates is also important to avoid direct contamination. By implementing these robust biosecurity and sanitation measures in addition to the strategic use of anticoccidial medications and vaccines, producers can significantly reduce the risk of coccidiosis outbreaks and the associated production losses and animal welfare concerns. The specific preventive strategies employed will depend on factors like the animal species affected the prevalence of coccidiosis in the particular production setting, and the resources and tools available to the producer or caretaker. The goal of these preventive measures is to create an environment and immune status in the animals that is unfavorable for the proliferation and transmission of the coccidial parasites thereby averting the clinical disease and its detrimental consequences.

2.4 THE ROLE OF ETHNOBOTANICALS IN MANAGEMENT OF COCCIDIOSIS

Traditional approaches to managing coccidiosis have relied heavily on the use of synthetic anticoccidial drugs and vaccines. However the growing concerns over anticoccidial resistance as well as consumer demand for more natural and sustainable production method have driven increased interest in exploring alternative and complementary solutions. Ethnobotanicals or plant-based traditional medicines have emerged as a promising avenue for the management of coccidiosis. Two such ethnobotanicals that have gained attention for their potential in parasitic diseases control are cloves *(Syzygium aromaticum*) and pomegranate (*Punica granatum*). Cloves prized for their distinctive aroma and flavor have long been used in traditional medicine systems for their medicinal properties. Recent scientific investigations have revealed that cloves possess potent antimicrobial, antioxidant and immunomodulatory activities which could be particularly beneficial in the context of coccidiosis management. Studies have demonstrated the ability of clove extracts and their active compounds such as eugenol which may directly inhibit the growth and development of *Eimeria* parasites. In vitro experiments have shown that clove-derived compounds can disrupt the lifecycle of coccidian oocysts preventing their maturation and subsequent infection of the host. Furthermore cloves have been found to enhance the host's immune response stimulating the production of protective cytokines and antibodies against which may in this study acct against coccidiosis.

Pomegranate another extensively studied ethnobotanical has also shown promise in the management of coccidiosis. Pomegranate and its bioactive compounds including punicalagins and ellagitannins have demonstrated significant antiprotozoan activity both in vitro and in vivo. These compounds have been found to interfere with the invasion, proliferation and transmission of most protozoan parasites thereby disrupting the parasite's life cycle and reducing the severity. In addition to their direct antiparasitic effects, pomegranate-derived compounds have also been shown to possess potent antioxidant and anti-inflammatory properties. These characteristics can help mitigate the damaging effects of coccidiosis on the host's intestinal health and overall wellbeing ultimately improving the animal's resilience and productivity.

The integration of cloves and pomegranate into coccidiosis management strategies can take various forms. These ethnobotanicals can be incorporated into animal feed or drinking water, or they can be administered as dietary supplements or herbal extracts. Additionally, they can be used in conjunction with other prevention and control measures such as improved sanitation, vaccination and the judicious use of conventional anticoccidial drugs to create a more holistic and sustainable approach to coccidiosis management. As the demand for natural and eco-friendly solutions in animal production continues to grow, the potential of cloves, pomegranate, and other ethnobotanicals in the management of coccidiosis has become increasingly relevant. Further research and field-based evaluations are necessary to fully understand the optimal application and integration of these plant-based remedies into comprehensive coccidiosis control programs.

The biological properties of extracts, antioxidant, anticancer, anti-inflammatory, antiparasitic among other properties obtained from several parts of pomegranate is reported in the present work. Due to such properties, the extracts have been used in therapeutics such as in the prevention of infection, inflammation, cancer, among other applications. However, other aspects are also referred in the present work such as the good practices of culture and fruit preservation search of new compounds and selection of cultivars through biotechnological techniques for obtaining juice or fruits ready to eat (Maria et al, 2010).

2.4.1 *Punica granatum*

The pomegranate (*Punica granatum*) is a plant species that is native to Iran and the surrounding countries in the central Asia region. Over time it has gradually expanded its distribution to areas such as the Himalayan region, the Eyalet of Anatolia and the broader Middle East (Saidi et al., 2009). *Punica granatum* commonly known as the pomegranate is a well-established member of the Punicaceae family. The pomegranate plant has glossy leaves and its flowers can be red, white, large or variegated in color. These flowers have tubular-shaped calyxes that eventually develop into the fruit (Samira et al., 2021). The pomegranate fruit itself is grenade-shaped, with a deep red, leathery skin and a crown-shaped calyx at the top. Inside, the seeds are surrounded by a small amount of tart, red juice and they are separated by a white, membranous pericarp (Samira et al., 2021). Pomegranates have long been recognized for their therapeutic properties and are considered a rich source of antioxidants, vitamins and minerals.

P. granatum has a long history of use in traditional medicine for treating various conditions. It has been used to address diarrhea, dysentery, hemorrhoids, intestinal parasites, sore throat, diabetes, nosebleeds and vaginal itching and is believed to have tonic effects on the heart (Askawi et al., 2018). In more recent times pomegranate has been explored for the treatment of numerous diseases including diabetes, Alzheimer's disease (Almuyawi et al., 2020), cancer, arthritis, male infertility, obesity and cardiovascular disorders (Dwang et al., 2018). Pomegranate is recognized as a primary source of beneficial ingredients, such as flavonoids, magnesium, potassium and iron. It also contains antioxidant components like alpha-linolenic acid, linoleic acid and oleic acid (Alkhatib, 2021).

In vitro studies have shown that the aqueous extract of pomegranate peels can have effective anti-parasitic properties against *Eimeria.* Consumption of pomegranate juice may therefore be a therapeutic approach against *Eimeria* infections (Singh et al., 2019). The antioxidant activity of pomegranate fruit can be attributed to the presence of compounds like ascorbic acid and phenolic compounds including punicalagin, punicalin, gallic acid, ellagic acid and anthocyanins. The antioxidant levels are highest in newly formed fruits and tend to decline as the fruit matures due to a reduction in ascorbic and phenolic acid levels (Singh et al., 2019). Diverse extracts of pomegranate fruit peels particularly the 80% methanolic extract have been found to be potent inhibitors of the parasite *Eimeria stiedae* (Singh, 2018).

The pomegranate plant been traditionally used in its entirety with various parts like the flowers, leaves, bark of young shoots, roots, fruit peel and pomegranate sauce being utilized. All components of the *Punica granatum* fruit which are abundant in tannins exhibit relatively strong astringent effects. Traditional medicine has employed several infusions or decoctions of the plant's flowers to treat simple diarrhea, vaginal discharge and to help relieve pancreas inflammation when accompanied by pomegranate peel. *Punica granatum* fruit juice is recommended to help heal gallbladder diseases. The fruit contains strong tannins which are considered a bitter nutrient. Its decoction appears to be helpful for treating various conditions such as ordinary diarrhea, dysentery and inflammation. The different chemical compositions of the pomegranate seeds, peels, juice, flowers, and leaves contribute to the fruit's overall benefits as each compound has its own beneficial functions.The therapeutic activities of *Punica granatum* have been attributed to compounds like delphinidin, gallocatechins, cyanadin, pelargonidin, ellagic acid, gallic acid and sitosterol.

The pomegranate peels consist of useful components such as ellagitannins, proanthocyanidin compounds, flavonoids, and minerals like magnesium, potassium, phosphorus, sodium and alkaloids.The seeds contain essential oils, triacylglycerols, octadecatrienoic fatty acids, lignins, hydroxycinnamic acids, gallic acid, protein, crude fibers, stearic acid, vitamins, punic acid, phytoestrogens like coumestrol and alkaloids.The fruit's leaves have tannins, flavones like apigenin and naringin, as well as calcium, iron, punicalagins, punicafolina, potassium, zinc, magnesium, saponins, and anthocyanins.The flowers and juice consist of compounds like ursolic acid, terpenic compounds, maslinic acid, asiatic acid, tannins, catechin, oleanolic acid, water, sugars, pectin, polyphenols, fatty acids, minerals, sterols, quercetin and ellagitannins.

Antiparasitic Activity of pomegranate

Parasitic diseases and infections pose a significant global threat, especially in developing and overcrowded regions particularly in African countries. These parasitic diseases can hinder economic development by impeding agricultural progress, which is a crucial income-generating activity in such countries. Over time the widespread use of chemical medication has led to an increase in parasite resistance to these treatments. However the utilization of medicinal plants has made a substantial difference and change worldwide in treating a variety of diseases across all continents. Khorrami and colleagues investigated the effect of pomegranate (*Punica granatum*) on the parasite *Eimeria*. Pomegranate has emerged as a promising alternative in the treatment of parasitic diseases, offering a potential solution to the growing problem of drug resistance.

Classification accccording to (USDA, 2023).

Kingdom Plantae

Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Punicaeceae
Genus	*Punica*
Species	*granatum*

Figure 3 Punica granatum image

Fig 2.3 *Punica granatum* image(Xhuveli, 2012).

2.4.2 *Syzygium aromaticum*

Syzygium aromaticum a member of the Myrtaceae family and is highly valued in medicine for its carminative and stimulant properties and is considered a natural anthelmintic (deworming agent). It is used extensively in Europe and Asia and is sometimes smoked in a type of cigarette known as "kretek" in Indonesia and is occasionally mixed with marijuana in coffee bars in the West to create marijuana spliffs. *Syzygium aromaticum* is used in medicine for its antibacterial, antiseptic and antibiotic properties. It has also been successfully used for the oral treatment of asthma and various allergic disorders. Furthermore the sesquiterpenes found in clove have been investigated as potential anti-carcinogenic (anti-cancer) agents as reported by Monnica et al., (2010). One notable use of clove is its ability to increase growth in poultry. Studies have demonstrated the potent antiparasitic effects of clove which can be attributed to the active compounds present in its buds.

The inhibitory activity of clove against parasites is primarily due to the presence of several key constituents including eugenol, eugenyl acetate, β-caryophyllene, hidroiphenyl, acetyl-eugenol, α-humulene, methyl salicylate, iso-eugenol, methyl-eugenol, phenylpropanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnetin, myricetin, gallic acid, ellagic acid and oleanolic

acid. These compounds possess the ability to denature proteins and react with cell membrane phospholipids, altering their permeability and consequently disrupting the parasites' cellular functions. Furthermore the flavonoids present in cloves have the potential to control coccidiosis a parasitic disease that can significantly impact poultry production. Flavonoids are known for their ability to reduce oxidative stress which is a crucial factor in the development and progression of coccidiosis. Additionally these phytochemicals can penetrate the cell membranes of parasites leading to the death of sporozoites and oocysts, the infective stages of the parasite. The versatility of clove in promoting poultry growth and exhibiting potent antiparasitic effects highlights its importance as a natural and effective alternative to conventional pharmaceutical interventions.

Cloves contain a powerful anesthetic compound called eugenol which also serves as an effective antiseptic in fighting bacteria and parasites that may cause infections. This is why cloves have proven to be highly effective in combating dental cavities leading to their common inclusion in oral healthcare products such as toothpaste and mouthwash.Beyond their dental applications cloves also possess essential oils that can be used to attack Eimeria oocysts, the infectious stages of the parasites that cause coccidiosis in animals. These essential oils are highly potent antioxidants and immunomodulators meaning they can regulate the immune system. The essential oils in cloves can stop the sporulation or reproductive cycle of *Eimeria* oocysts by penetrating their protective walls. This direct action on the parasites' life cycle is a key reason why clove-derived essential oils have been widely used to control coccidiosis. Additionally the essential oils in cloves can help reduce the clinical signs and symptoms of coccidiosis due to their both direct and indirect anticoccidial activities. This makes cloves a valuable natural alternative in the management of this parasitic disease which can have significant impacts on animal health and productivity. The diverse applications of cloves from their use in oral care to their potential in controlling coccidiosis, highlight the versatility and efficacy of this plant-based resource. The multifaceted properties of clove particularly its anesthetic, antiseptic and antiparasitic capabilities make it a promising candidate for further exploration and utilization in various healthcare and agricultural domains.

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium
Species	*aromaticum*

Fig 2.4 Image of *Syzygium aromaticum* (Petruzello, 2024).

Figure 4 Syzygium aromaticum Image

CHAPTER 3

METHODS AND MATERIALS.

3.1 STUDY AREA.

The research project was carried out from October 2023 to February 2024 in Harare at the Central Veterinary Laboratories in Zimbabwe.

3.2 COLLECTION OF *P. GRANATUM* AND *S. AROMATICUM* SAMPLES

Mature *P. granatum* fruits were obtained from mature local pomegranate fruit trees. The *P.granatum* were purchased from local herb supermarket

Healthy day old broiler chicks were purchased from a local agri-shop. The chicks were reared until they were 21 day old. The chicks were caged in a fowl run each in its apartment and were treated accordingly. Good sanitation and condition for growth were provided and to prevent other diseases and

microbe infection before the experiment is carried out. Same nutritional and management conditions were given.

3.3 PREPARATION OF *PUNICA GRANATUM* EXTRACTS

Pomegranate peels were separated from the whole edible fruit and washed with water. The pomegranate peels were dried in the shade in an open space subjected to air. Peels were left for about 7days and were weighted until there was insignificant change in mass of the moisture content or until completely dry. The dried peels were grinded using a pestle and mortar to a coarse powder and stored in an airtight container under room temperature. 100g of the ground pomegranate peels was weighted and soaked in the three solvents in the (50:50) ratio. To prepare samples, ground pomegranate peels were separately soaked in solvents of ethanol, methanol and water using a ratio of 50:50 (sample :solvent) at room temperature for 24 hours. After 24 hours, the samples were filtered with filter paper (Whatman No. 2 paper) and the filtrate was stored. Most of the published literature about the different extraction methods/solvents recommend that the methanol extraction method is better than others in terms of the abundance of phenolic compounds and increase antioxidant activity(Kharchoufi et al, 2018).

3.4 PREPARATION OF *SYZYGIUM AROMATICUM* EXTRACTS.

The cloves were dried under the shade for several days. They were placed on clean mats in an open space so that air could pass through the cloves. They were turned frequently to ensure they develop an even brown color. The color of the buds changed from pale russet to a darker brown as the clove dries. The drying process took about five to eight days. After drying, the cloves were ground into a coarse powder using a mortar and pestle. Three solvents methanol, ethanol and distilled water were used for the extraction. 50 grams of the ground sample was soaked in 250ml of different solvents water, methanol (80%) and ethanol(70%) for 72hrs.After 24 hours the samples were subjected in a shaking water bath and were filtered using Whatman filter paper and the filtrate was stored for analysis.

3.5 PREPARATION OF *EIMERIA*.

Eimeria species were collected at the parasitology laboratory of the veterinary from infected chicken which were ready to be sacrificed and incinerated. Samples of stool were collected in an airtight plastic by use of spatulas. The *Eimeria* species were prepared using the fecal method below.

3.5.1 IDENTIFICATION OF *EIMERIA* SPECIES.

The *Eimeria* species were detected using the fecal flotation method.

The sample was collected was labeled and their cylinder respectively. 42ml of water was added in the cylinder and 3 grams of fecal sample. The solution was mixed well using a sterilized applicator stick. The solution was transfered in a testtube 1cm from the top. The remaining 1mm was filled with salt (NaOH) creating a meniscus. A coverslip was placed on top and the eggs were allowed to float to the top and stick to the coverslip. After 10 minutes the coverslip was placed on top of the microscope slide. The sample was viewed on a light microscope and the eggs were identified, measured and examined under the 10x lens.

3.6 STUDY DESIGN

In the study the chicks were randomly devided into 4 groups. All chickens were reared under the same management conditions and were given the same feeding. *Eimeria* species were orally inoculated in saline water in young broilers. The groups were monitored and as soon as the broilers showed symptoms of the infection like bloody stool in the faeces, fecal samples were collected in all infected groups and examined for oocyst per gram (OPG) of the faeces. Group 1 and 2 were the ones infected with the *Eimeria* whilst group 3 received Amporium a standardized drug and group 4 is the negative control with no treatment. The fecal samples were examined for confirmation of the infection using the Mc master method to see the quantity of the oocyst because the fecal flotation technique is best for qualitative analysis.

Crude extracts of *Syzygium aromaticum* and Punica granutum of different concentration were then administered to the groups which were infected. Within a period of 1 week samples were collected and analyzed for the efficacy assessment of the crude herbal plant species (Gadzirai, 2021). The first group was administered with the *Punica granutum* extracts, the second group was given the *Syzygium aromaticum* and the third group was given the standardized drug Ampolium. The effect of PPE and the *Syzygium aromaticum* extracts were examined after every 7 days in the 28 day experimental period. Examination was on weight gain and oocysts counts. At the end of the experiment the fecal samples were collected for the final analysis of the OPG. The Mc Master method was used for this analysis.

3.6.1 McMASTER TECHNIQUE.

To prepare samples in the Mc Master method 42ml of water was added in the graduated cylinder. The collected fecal samples were mixed using a rod. 3ml of fecal samples were added in the cylinders respectively resulting in a volume of 45ml. the samples were mixed thoroughly. The mixtures were then sieved using a tea strainer and the tea strainer was cleansed after straining a fecal mixture to avoid contamination of another sample. The filtrates were collected each sample in its own beaker and the waste was discarded in the discarding bin. The eggs are in the filtrate solution.

The egg suspensions were poured in 15ml centrifugation test tubes and filled to approximately 1ml from the top. To concentrate the eggs, samples were centrifuged for 4 minutes at 1500rpm.

The supernatants were decanted in the discarding bin and a flotation solution (NaOH) was added and the tubes were half filled. The tubes were stirred using an applicator stick.

Parafilms were placed on top of the testubes and the samples were mixed invertedly to evenly mix the egg solutions. The Mc Master slides were washed to make it easy for the solution to enter the chambers.

Using a Pasteur pipette both chambers for each sample solution was filled with the solution.

Each of the chambers were viewed under a light microscope under 10x lens to count and identify the all the eggs.

All the readings were calculated as OPG= A+Bx 50

Evaluation: Various parameters such as oocyst count from chicken droppings, weight gain, and mortality rate was monitored throughout the study.

3.7 LESION SCORING

After all the experiments all the chickens were collected and were taken to the slaughter house for sacrifice for lesion scoring. Chicken intestines were collected from the slaughterhouse all labeled according to the treatments they had been subjected to. The intestines were rinsed using saline solution and were then fixed using formalin for 48hours. The intestines were cut using the scissors and forceps. They were cut longitudinally and the mucosal surface was examined. A microscope was used to examine the severity, size and number of inflamation and necrosis.

Results were recorded accordingly.

3.8 DATA ANALYSIS

The mean oocyst counts that were calculated before and after the administration of the different extracts were used to evaluate percentage in reduction or reduction rate, using the formula below:

Reduction rate = ((Initial oocyst counts-Final oocyst counts) 100)/ Initial count.

The effectiveness of the treatments was also done by comparing the percentage difference of oocytes count. The data was entered into Microsoft Excel spread sheet and analyzed using SPSS 23 software version Analysis of variance (ANOVA) at $\alpha = 0.05$ level of significance. If there was a significant difference between the data, the Tukey method was utilized. The results of this study were considered as significant when a P value was less than 0.05.

CHAPTER 4

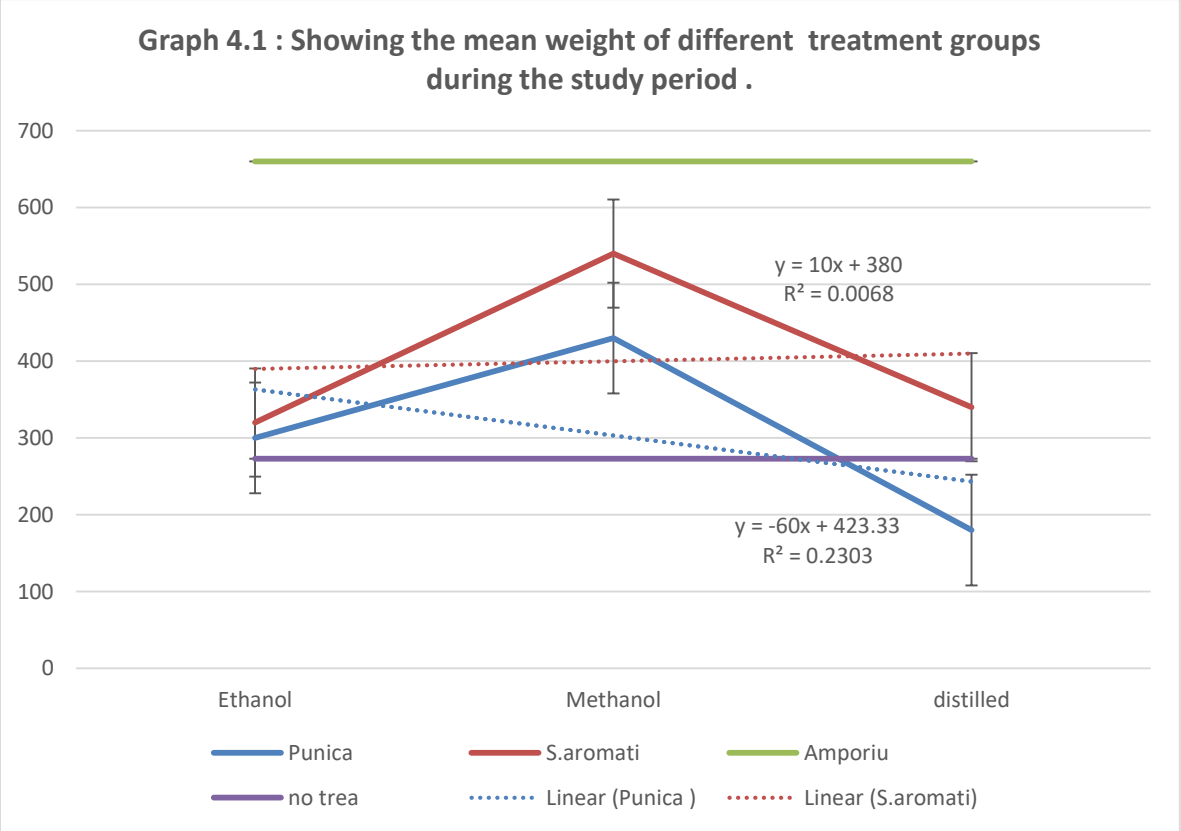
RESULTS, INTERPRETATION AND ANALYSIS

Table 4.1 Mean weight In grams of 4 groups of birds in 28days under different treatments

Table 1 Mean weigth in grams of 4 groups of birds in 28 days under different treatments

Group (3 birds in each treatment)	DAY	Ethanol extract	Methanol extract	Distilled
Punica granatum	7	370	350	460
	14	490	520	530
	28	670	780	640

Weight gain	(28 day weght-7 day weight)	300	430	180
Syzygium aromaticum	7	550	450	420
	14	648	780	640
	28	870	990	760
Weight gain		320	540	340
Amporium	**Day**			**Mean weight**
	7			400
	14			560
	28			1060
Weight gain				660
No treatment	**control**	**live**	**Dead**	**Mean weight**
	7	3	0	300
	14	2	1	270
	28	1	1	250
				273



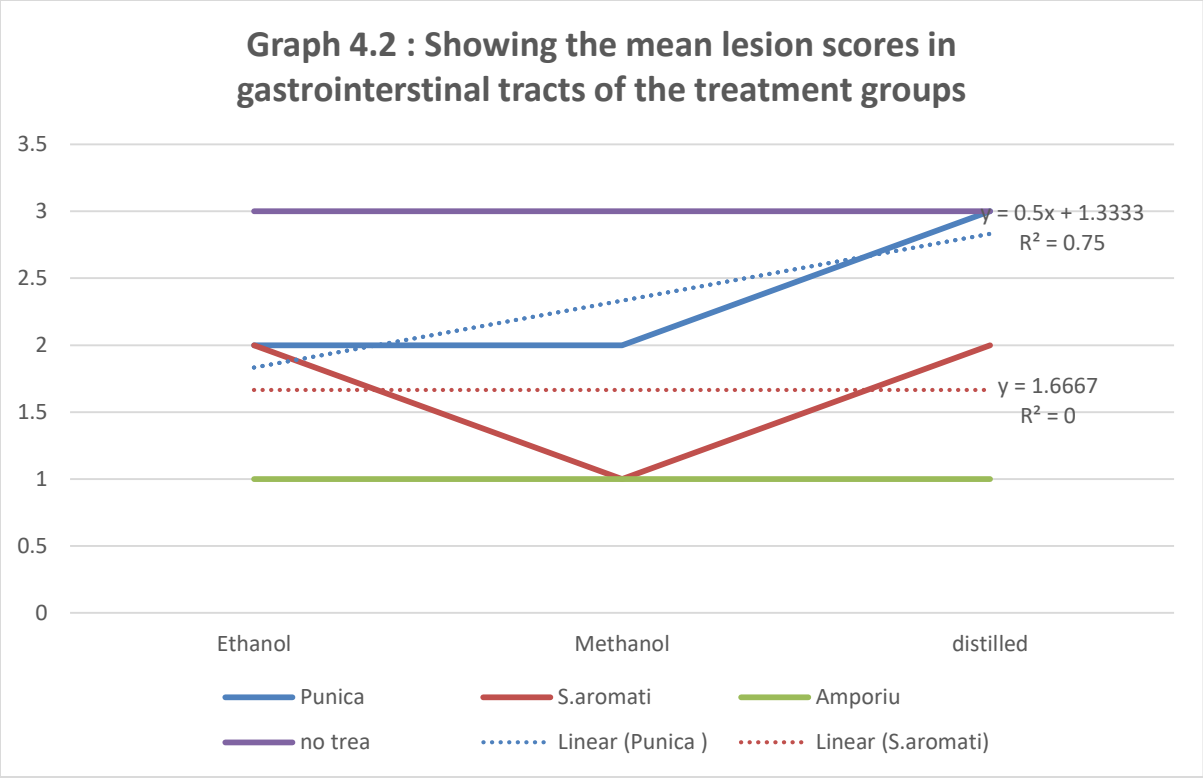
Graph 4.1 above Shows that *S.aromaticum* extracts have got more impact towards inhibition of Coccidiosis compared *Punica granatum.* Weight in grams was plotted against time in days.

Table 4.2 : Showing mean lesion scoring in different groups treatment groups

Table 2 Mean lesion score in different treatment groups

Group (3 birds in each)	**DAY**	**Ethanol extract**	**Methanol extract**	**Distilled**
Punica granatum	28	2	2	3
Syzigium Aromaticum	28	2	1	2
	Control	Group		
Amporium	28	1		
No treatment	28	3		

Table 4.2 above shows that there more lesion in distilled water extracts compared to ethanolic and methanolic extracts.

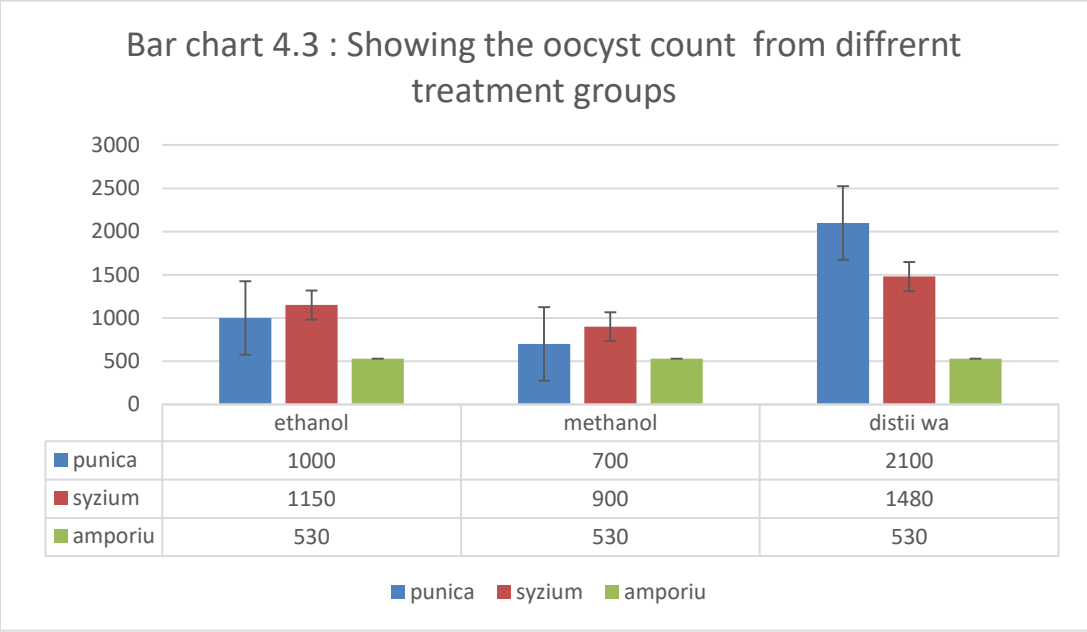


Graph 4.2 above shows that *S .aromaticum* impacted more lesion remedy effects compared to *Punica granatum* during the study.

Table 4.3: Showing the Oocysts counts per gram in each group chickens

Table 3Oocyts count per gram in each group chickens

Group	DAY	Ethanol extract	Methanol extract	Distilled
Punica granutum	7	5000	8000	11000
	14	2000	3000	7800
	28	1000	700	2100
Syzygium aromaticum	7	11600	7000	6000
	14	6000	5500	3020
	28	1150	900	1480
	Amporium	**Control Group**		
	7	7000		
	14	3000		
	28	530		
	No treatment	**Control Group**		
	7	11000		
	14	84000		
	28	127000		



Graph 4.3 :Shows that *Punica granatum* have got better impact in ethanolic extract and Methanolic extract than in distilled water extract and *Syzygium aromaticum* methanolic extracts were the best compared to ethanolic and distilled water extracts of the same plant species.

CHAPTER 5

5. DISCUSSION.

Coccidiosis has been a well-known issue in the poultry industry for many years, but it remains a major economic burden. According to research by Dee Gusta (2007), the significant economic damages caused by this parasitic disease include losses in animal performance, mortality, weight loss and reduced nutrient digestibility due to gut damage. In this study the use of crude herbal extracts from *Punica granatum* (pomegranate) and *Syzygium aromaticum* (clove) were used for the prevention and control of coccidiosis through oral treatment efficacy trials. The potential success of these plant-based treatments in managing poultry diseases provided a significant boost to poultry production by helping to address the challenges posed by the growing problem of antiparasitic drug resistance resulting from the frequent use of commercial pharmaceuticals. The study evaluated the use of *Punica granatum* and *Syzygium aromaticum* extracts as potential natural treatments, and the potential benefits of these plant-based approaches in overcoming the issues of antiparasitic drug resistance in the poultry industry.

5.1 Investigation of the impact of treatmemt effect *Syzygium aromaticum* and *Punica granatum* towards the mean weight improvement of coccidioscis infected chicken groups during the study

The introduction of antiparasitic treatments for infected chickens can improve their immune system, leading to better health status and increased average weight. According to Archaya (2017), coccidia parasites are commonly present in poultry operations but clinical disease only occurs when susceptible birds such as those that are immunosuppressed or have concurrent diseases, ingest relatively large numbers of sporulated oocysts. Both clinically infected birds and those that have recovered from coccidiosis can shed oocysts in their feces which can then contaminate feed, dust, water, litter and soil. These oocysts can be transmitted through equipment, personnel, the presence of insects (e.g., flies) and rodents. The sporulated oocysts can survive for extended periods depending on environmental factors, as noted by Adebe E and Gugsa (2018). According to the study results presented in Table 4.1, the introduction of crude herbal extracts from *Punica granatum* (pomegranate) *a*nd *Syzygium aromaticum* (clove) to infected chickens resulted in significant improvements in their weight compared to the untreated group.

The mean weight gain from day 7 to day 14 was greatest for the methanol extract of *Syzygium aromaticum* (540g), followed by the methanolic extracts of *Punica granatum* (430g), the ethanolic extracts of Syzygium aromaticum (320g) and the ethanolic extracts of *Punica granatum* (300g). In contrast, the distilled water extracts of both herbs showed relatively lower mean weight gains compared to the ethanolic and methanolic extracts.The trending models for the weight deviations indicate that *Syzygium aromaticum* ($Y=10x + 380$) had a greater efficacy against coccidiosis compared to *Punica granatum* ($Y=-60x + 423.33$) when using extracts of the same solvent.

The symptoms of coccidiosis can range from decreased growth rate and diarrhea to high mortality, decreased feed and water consumption, weight loss, and reduced egg production. While survivors of severe infections may recover within 10-14 days, they may never fully regain their original growth and production levels, as noted by De Gussem (2007).

5.2 Assesment of the gastro intestinal effects lesion scores efficacy evaluation of crude herbal extrcts of *Syzygium aromaticum* and *Punica granatum* against coccidiosis

Lesion scoring is a method used to interpret poultry coccidiosis by observing the intestinal tracts for macroscopically visible lesions. The lesions are scored on a scale of 0 to 4, where 0 represents a healthy intestine with no signs of infection, and 4 indicates extremely severe lesions caused by Eimeria parasites (Vallejch et al, 2011). This method is labor-intensive, occasionally subjective and requires the expertise of veterinarians or highly trained individuals to be reliable (Peek HW, Landman WJM 2011).

In the study the mean lesion score for the untreated group was 3, while the methanolic extracts of *Syzygium aromaticum* and *Punica granatum* had lesion scores of 1 and 2 respectively. The ethanolic extracts of both herbs resulted in an equal lesion score of 2 and the distilled water extracts of *Syzygium aromaticum* and *Punica granatum* also had a lesion score of 2. The amprolium control group had a lesion score of 1, indicating that the methanolic extracts particularly *Syzygium aromaticum*, exhibited the best efficacy against coccidiosis in this trial. The trendline model for the lesion scores of *Punica granatum* extracts ($Y=0.5x + 1.333$) was higher compared to that of *Syzygium aromaticum*, suggesting that *Syzygium aromaticum* could be a more effective herbal treatment and could improve the recovery of chickens infected with coccidiosis. The comparative lesion scores of the different herbal extracts and the control group indicates that *Syzygium aromaticum* may be a more promising herbal treatment for coccidiosis in chickens.

5.3 Impact assessment of the crude herbal extracts of *Syzygium aromaticum* and *Punica granatum* against Coccidioscis Oocysts counts

The impact of the crude herbal extracts was evaluated based on their ability to reduce the number of *Eimeria* oocysts. The results showed that the methanolic extracts exhibited the best improvement, followed by the ethanolic extracts, and finally the distilled water extracts.

The ANOVA analysis presented in Table 4.4a indicates that there were significant differences in the efficacy of the plant extracts against the infection at day 7 ($p=0.939$), day 14 ($p=0.790$), and day 28 ($p=0.854$). Similarly, the impact of the solvent type used for extraction, as shown in Table 4.4b, also had a significant effect on the results at day 7 ($p=0.954$), day 14 ($p=0.866$), and day 28 ($p=0.074$). These findings suggest that the choice of solvent used for extracting the phytochemicals played an important role in the prevention of coccidiosis infections by both *Punica granatum* and *Syzygium aromaticum*.

Furthermore, the post-hoc tests presented in Table 4.4c showed significant differences between the days when using the same crude plant herbal extract. This indicates that the efficacy of the extracts varied over the course of the study period. The methanolic extracts exhibited the best performance in reducing *Eimeria* oocysts, followed by ethanolic and distilled water extracts. The efficacy of the plant extracts and the choice of solvent used for extraction both had a significant impact on the results over time. The post-hoc tests revealed significant differences in the efficacy of the same crude plant extracts across different time points.

CHAPTER 6

6.1 Recommendation for Further studies

Although traditional methods such as lesion scoring and oocyst detection in feces are still being used, molecular biological methods are applicable in most research since the above mentioned traditional methods are time-intensive and in the case of detection of oocyst, it is hard to distinguish what kind of oocysts are there .According to Fatoba et al 2018 ,previous techniques used to distinguish different species were based on the isoenzyme patterns of oocysts and rRNA and rDNA. Polymerase chain reaction (PCR) is a rapid, accurate and highly sensitive molecular diagnostic technique to identify the *Eimeria spp*. in chickens by inspecting their variations of genomic DNA.The method enables amplification of the chicken coccidian species-specific DNA sequence for the detection and discrimination to hundreds of millions for a few hours and the new copies can be divided by electrophoresis to visualize it under UV light by a fluorescent dye (Fatoba et al 2018). Synergestic effects of *Punica granatum* and *Syzygium aromaticum* must be investigated using the same extraction methods and also the compunds must be screened of some of the phytochemicals which may be toxic to chickens.

6.2 Conclusion

The study provided a baseline assessment for the treatment of coccidiosis . The significance of *Syzygium aromaticum* and *Punica granatum* in eradiaction of coccidian have been recognised as a possibility upon proper solvent extraction and observation of the minimum dose required to counteract the disease effects. Coccidiosis is a major enteric parasitic disease in the poultry industry and is caused by seven *Eimeria* parasite species developing in a particular chick's digestive tract. These species induce symptoms from subclinical enteric infection to subacute mortality. For these reasons, coccidiosis is still mightily important to prevent economic loss worldwide. Although diverse control measures have been accomplished against

the disease, prophylactic use of anticoccidial drugs was an extensively used control approach which makes a problem of drug resistance recently. Hence, phytogenic compounds are emerging for the control and prevention of poultry coccidiosis instead of previous methods. Therefore, there need for various researches related to phytogenic compounds affecting coccidiosis in broilers.

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APPENDICES

Appendix 1 Extract tests of between subjects.

Analysis of Variance output from IBM SPSS ver 23 and Extract Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	day7	60000.000[a]	1	60000.000	.007	.939
	day14	493066.667[b]	1	493066.667	.081	.790
	day28	12150.000[c]	1	12150.000	.039	.854
Intercept	day7	393660000.000	1	393660000.000	43.935	.003
	day14	124397066.667	1	124397066.667	20.460	.011
	day28	8954816.667	1	8954816.667	28.520	.006
Extract	day7	60000.000	1	60000.000	.007	.939
	day14	493066.667	1	493066.667	.081	.790
	day28	12150.000	1	12150.000	.039	.854
Error	day7	35840000.000	4	8960000.000		
	day14	24320266.667	4	6080066.667		
	day28	1255933.333	4	313983.333		
Total	day7	429560000.000	6			
	day14	149210400.000	6			
	day28	10222900.000	6			
Corrected Total	day7	35900000.000	5			

day14	24813333.333	5			
day28	1268083.333	5			

a. R Squared = .002 (Adjusted R Squared = -.248)
b. R Squared = .020 (Adjusted R Squared = -.225)
c. R Squared = .010 (Adjusted R Squared = -.238)

Appendix 2 Tests of between- subject effects

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	day7	1120000.00[a]	2	560000.000	.048	.954
	da14	2264133.33[b]	2	1132066.667	.151	.866
	day28	1044633.33[c]	2	522316.667	7.013	.074
Intercept	day7	393660000.0	1	393660000.0	33.956	.010
	da14	124397066.7	1	124397066.7	16.550	.027
	day28	8954816.667	1	8954816.667	120.226	.002
solvent	day7	1120000.000	2	560000.000	.048	.954
	da14	2264133.333	2	1132066.667	.151	.866
	day28	1044633.333	2	522316.667	7.013	.074
Error	day7	34780000.00	3	11593333.33		
	da14	22549200.00	3	7516400.000		
	day28	223450.000	3	74483.333		
Total	day7	429560000.0	6			
	da14	149210400.0	6			
	day28	10222900.00	6			
Corrected Total	day7	35900000.00	5			
	da14	24813333.33	5			
	day28	1268083.333	5			

a. R Squared = .031 (Adjusted R Squared = -.615)
b. R Squared = .091 (Adjusted R Squared = -.515)
c. R Squared = .824 (Adjusted R Squared = .706)

POST HOC TESTS

Appendix 3 Post Hoc tests

Multiple Comparisons

Dependent Variable		(I) solvent	(J) solvent	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
day7	LSD	distilled water	Ethanol	1000.00	3404.898	.788	-9835.91	11835.91
			methanol	200.00	3404.898	.957	-10635.91	11035.91
		Ethanol	distilled water	-1000.00	3404.898	.788	-11835.91	9835.91
			methanol	-800.00	3404.898	.829	-11635.91	10035.91
		methanol	distilled water	-200.00	3404.898	.957	-11035.91	10635.91
			Ethanol	800.00	3404.898	.829	-10035.91	11635.91
	Bonferroni	distilled water	Ethanol	1000.00	3404.898	1.000	-15536.42	17536.42
			methanol	200.00	3404.898	1.000	-16336.42	16736.42
		Ethanol	distilled water	-1000.00	3404.898	1.000	-17536.42	15536.42
			methanol	-800.00	3404.898	1.000	-17336.42	15736.42
		methanol	distilled water	-200.00	3404.898	1.000	-16736.42	16336.42
			Ethanol	800.00	3404.898	1.000	-15736.42	17336.42
da14	LSD	distilled water	Ethanol	1160.00	2741.605	.701	-7565.01	9885.01
			methanol	1410.00	2741.605	.643	-7315.01	10135.01
		Ethanol	distilled water	-1160.00	2741.605	.701	-9885.01	7565.01
			methanol	250.00	2741.605	.933	-8475.01	8975.01
		methanol	distilled water	-1410.00	2741.605	.643	-10135.01	7315.01
			Ethanol	-250.00	2741.605	.933	-8975.01	8475.01
	Bonferroni	distilled water	Ethanol	1160.00	2741.605	1.000	-12155.04	14475.04
			methanol	1410.00	2741.605	1.000	-11905.04	14725.04
		Ethanol	distilled water	-1160.00	2741.605	1.000	-14475.04	12155.04
			methanol	250.00	2741.605	1.000	-13065.04	13565.04
		methanol	distilled water	-1410.00	2741.605	1.000	-14725.04	11905.04
			Ethanol	-250.00	2741.605	1.000	-13565.04	13065.04
day28	LSD	distilled water	Ethanol	990.00*	272.916	.036	121.46	1858.54
			methanol	715.00	272.916	.079	-153.54	1583.54
		Ethanol	distilled water	-990.00*	272.916	.036	-1858.54	-121.46
			methanol	-275.00	272.916	.388	-1143.54	593.54
		methanol	distilled water	-715.00	272.916	.079	-1583.54	153.54
			Ethanol	275.00	272.916	.388	-593.54	1143.54
	Bonferroni	distilled water	Ethanol	990.00	272.916	.108	-335.46	2315.46
			methanol	715.00	272.916	.237	-610.46	2040.46
		Ethanol	distilled water	-990.00	272.916	.108	-2315.46	335.46
			methanol	-275.00	272.916	1.000	-1600.46	1050.46
		methanol	distilled water	-715.00	272.916	.237	-2040.46	610.46
			Ethanol	275.00	272.916	1.000	-1050.46	1600.46

Based on observed means.
The error term is Mean Square(Error) = 74483.333.

*. The mean difference is significant at the .05 level.

Appendix 4 Analysis Of Variance

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
methanol	Between Groups	73958755.556	2	36979377.778	6.981	.027
	Within Groups	31782600.000	6	5297100.000		
	Total	105741355.556	8			
Ethanol	Between Groups	65873755.556	2	32936877.778	40.315	.000
	Within Groups	4901933.333	6	816988.889		
	Total	70775688.889	8			
distill	Between Groups	65947622.222	2	32973811.111	6.477	.032
	Within Groups	30546866.667	6	5091144.444		
	Total	96494488.889	8			

Appendix 5 Comparative analysis of solvent to herbal effectiveness

Comparative analysis of solvent to herbal effectiveness Output summary from IBMSpss ver 23

Dependent Variable	Group	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
methanol	ampor	3510.000[a]	1041.733	617.685	6402.315
	punica	2666.667[a]	1041.733	-225.648	5558.981
	syz	7281.638[a]	1362.985	3497.385	11065.892
	zyz	4186.723[a]	2043.353	-1486.534	9859.980
Ethanol	ampor	3510.000[a]	748.907	1430.700	5589.300
	punica	3900.000[a]	748.907	1820.700	5979.300
	syz	4495.763[a]	979.857	1775.243	7216.282
	zyz	4408.475[a]	1468.977	329.941	8487.009
distill	ampor	3510.000[a]	600.758	1842.029	5177.971
	punica	6966.667[a]	600.758	5298.696	8634.638
	syz	2416.215[a]	786.021	233.871	4598.559
	zyz	5667.571[a]	1178.383	2395.856	8939.286

a. Covariates appearing in the model are evaluated at the following values: day = 16.33