MAKERERE UNIVERSITY

INDIGENOUS KNOWLEDGE, EFFICACY AND SAFETY OF MEDICINAL PLANTS USED IN CONTROL OF CHICKEN HELMINTHS IN UGANDA: A CASE OF SOROTI DISTRICT

BY

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DECLARATION AND APPROVAL

I **Gerald Zirintunda,** hereby declare that this dissertation is my original work and that it has never been submitted elsewhere for any kind of academic award or publication. Published articles from this dissertation are listed on page xv, Secondary data and literature sources used throughout the disseratation were duely accredited to the original authors. I also certify that this work has been subjected to antiplagiarism check confirming that no part of this book may be attributed to other previous scholarly works.

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DEDICATION

This dissertation is dedicated to my children Ainza Elizabeth Gweyawa, Kagaiga Jesse, Fugga Gloria, Kaganzi Judith and Lwigule Marvellous Joanna.

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LIST OF ABBREVIATIONS AND SYMBOLS

µg	micrograms
µL	microliters
AMR	Antimicrobial Resistance
EC_{50}	Effective concentration that can give half the maximum response
Ecg:	Egg count per gram of faeces
FCR	Food conversion ratio
FECRT	Faecal Egg Count Reduction Test
FGD	Focus group discussion
g	grams
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
IACUC	Institutional Animal Care and Use committee
IRB	Institutional Review Board
IU	International units
Kg	kilograms
mg	Milligrams
ml	Millilitres
Na^+	Sodium ions
^oC	degrees centigrade
PCR	Polymerase Chain Reaction

PCV Packed cell volume

SPE Supercritical Fluid extraction

SPSS Statistical Package for the Social Sciences

UAE Ultrasonic assisted extraction

LIST OF PUBLICATIONS AND MANUSCRIPTS

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3. Zirintunda, G., Kateregga, J., Nalule, S. *et al.* Extracts of *Carica papaya* L. and *Capsicum annuum* L. showed comparable efficacy to piperazine citrate and levamisole hydrochloride in treatment of poultry helminths. *Beni-Suef Univ J Basic Appl Sci* **14**, 25 (2025). https://doi.org/10.1186/s43088-025-00607-z

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1. Ethnoveterinary Practices for chicken helminths control in the communities of Soroti district, Uganda
2. Implications of knowledge, attitudes and practices on the use of ethnoveterinary medicine in the control of chicken diseases among the chicken owners in Soroti district, Uganda

Policy briefs from this thesis;

1. Guideline on the use of Ethnoveterinary Medicines in chickens

ABSTRACT

This study aimed at assessing the indigenous knowledge, phytochemistry, efficacy and safety of selected medicinal plants used in the control of chicken helminths in Soroti district. Indigenous knowledge was studied using focus group discussions and farmer interviews. The knowledge, attitudes and practices of ethno-veterinary utilization in chickens was done among 407 chicken owners of 20-50 years using structured questionnaires and focus group discusions. Qualitative and quantitive pytochemiical analyses were carried out using standard procedures and GC-MS, respectively. Efficacy determination of the crude extracts from selected plants was done by *in-vitro* adult *Ascaridia galli* paralysis studies and *in-vivo* by faecal egg count reduction (FECR) assessment in chicken. The toxicological effects of the extracts on chicken were evaluated using haematological, biochemical and histopathological evaluations. Twenty-one indigenous seven-week-old male chickens were fed on feeds from Nuvita® feeds Uganda limited and *adlib* tap water. They were divided into seven groups of three chicken per group. *Carica papaya* leaves ethanol and acetone extracts (CPLe and CPLa)), *Capsicum annuum* ripe fruits ethanol extract and acetone extracts (CAFe and CAFa), levamisole HCl, Piperazine citrate and phosphate buffered saline (PBS). All plant extracts were given at dose of 0.48g per bird as determined from a previous *in-vitro* experiments. Levamisole HCl was given at 25mg/kg body weight and piperazine citrate at 100mg/kg body weight. The control group received 0.2% DMSO in PBS. About 28 plant families with 39 species were mentioned. The most ranked plants were *Capsicum annuum* L. (PRK 65.4%) followed by *Carica papaya* L. (PRK 42.3%). Leaves were most used, were pounded and herbs orally administered. The mean knowledge score was 11.6/16 (SD=3.5). The mean attitudes score was 7.8/10 (SD=1.95). The mean practices score was 16.39/32 (SD= 5.58). The standardized mean scores were 72.5, 78 and 51.2 for knowledge, attitudes and practices respectively. Age was significantly related to knowledge, attitudes and practices ($p < 0.05$, $p < 0.001$, $p < 0.001$) respectively. GC-MS analysis of *Carica papaya* L of acetone extracts contained vitamin C (42%) and sterols (13%), whereas the ethanol extracts contained lipids (45.04%) and pyranones (20.3%). The acetone extracts of *Capsicum annuum* L had lipids (45.04 %) and alkanes (27.7%), whereas ethanol extracts exhibited lipids (50.16%) and alkaloids (22.73%). About 0.08g/ml of the extracts had paralyzed more than 50% of adult *A. galli* after 5 hours compared to the lower concentrations. On

average, FECR ranked as follows: levamisole hydrochloride>CPLa>CAFa>CAFe>CPLe>piperazine citrate with the percentage reductions ranging from 98.67±2.309 -35.67±2.082, respectively. Biochemically, CPLe, CAFe and piperazine citrate caused significantly higher blood sodium than CAFa (*p=0.046, p=0.005, p=0.04*), respectively. CPLe caused more serum albumins and levamisole caused higher AST levels than CAFe (*P=0.02, p=0.04*), respectively. CAFe, CPLa and levamisole caused eosinophilia compared to the PBS (*p= 0.01, p= 0.017 and p= 0.001*) respectively. CAFa and piperazine caused eosinopenia compared to PBS *(p=0.000)* for each. Except CPLe, all extracts caused various levels of inflammation in the kidney and liver in the order of CAFe > CPLe > CAFa.

In conclusion, the extracts were effective but CPLa was more efficacious and comparable to levamisole hydrochloride. All extracts were more efficacious than piperazine citrate at its recommended therapeutic dose. All extracts were toxic except CPLe. The study shows that extracts are not safer than synthetic anthelmintics and therefore they should also be used with caution**.**

CHAPTER ONE

INTRODUCTION

1.1 Background

Use of plant medicines in animals is as old as human existence but use records can be traced from the beginning of the 20th century (Gaoue et al., 2017; González & Vallejo, 2021). Natukunda et al. (2011) found the use of ethnoveterinary practices (EVP) at 66% in Kamuli plains of Uganda, possibly because there are many plants with therapeutic action (Zirintunda et al., 2022). The local ethnoveterinary knowledge has not been widely documented nor scientifically tested despite its advantages of being cheap, organic, acceptable and sustainable (Byaruhanga et al., 2015). Ethnoveterinary approaches have minimal effects on ecosystems (Byaruhanga et al., 2015; Eshetu et al., 2015). Sometimes farmers use EVP because they lack access or cannot afford conventional drugs (Eiki et al., 2021; Prakash et al., 2021; Suroowan et al., 2017).

Ethnoveterinary practices have provided various opportunities even in the affluent Europe (José Antonio González et al., 2019; Mattalia et al., 2021). However, there are controversies and obscurities in ethnoveterinary medicine (Gakuya et al., 2020). Various ethnoveterinary compounds could be very toxic (Abdel-Daim et al., 2018), since their phytochemical profiles are unknown. The phytochemical profile of particular species varies with region and season. Ethnoveterinary knowledge is usually mingled with fallacies and myths that requires empirical proof (Oda et al., 2024). Ethnoveterinary knowledge is usually not explicitly shared in all fora (Sibanda and Chiuta, 2018) , therefore may have errors unless proven. Most research of action of plant helmintics focusses on livestock, current literature of actions in chicken is meagre. Some anthelmintic researchers have made conclusions based on models or only *in vitro* assays missing the *in vivo* assays that reflect the chicken-helminths interactions with the drugs (Islam & Jahan, 2020).

Poultry is a key enterprise in the developing world (Attia et al., 2022). Chickens make the biggest percentage of poultry in Uganda with the indigenous breed being majority (UBOS, 2019). In Uganda, the poultry sector is hindered by disease parasites and lack of effective acceptable interventions against them (Byaruhanga et al., 2017). This has

become more stifling as farmers try to transform from traditional subsistence to commercial poultry farming (MAAIF, 2019). Although generally categorized as indigenous, chickens in Uganda exhibit great phenotypic variability almost in all characters for the various geographical regions (Yussif et al., 2023). Ugandan's chickens population was 37.4 million by 2008, 35.5 million in 2018 (UBOS, 2019) and was 57.8 million in 2021 (National Livestock Census, 2021); generally unsteady increase in numbers was realized.

At least each homestead in the rural and semi-urban Soroti owns chickens. In rural Soroti district; many households usually cluster together in a single homestead with usually very distant neighbouring homesteads. Each household runs their own stocking programs although other management practices are collectively done in the homestead. Soroti is among the leading districts in rearing indigenous chickens in Uganda, birds are raised on a free-range system (Soroti District Production Department, 2021). Local chickens in Uganda usually are on free range and scavenge for food (Mwesigwa et al., 2015), which predisposes them to many parasites. The biosecurity systems in the traditional chicken systems are usually very low if at all (Ouedraogo et al. 2024). Chicken numbers in Soroti district would be greatly improved with better health management (Attia et al., 2022).

The economic impacts of chicken helminths have not been explicitly studied, however helminths generally reduce production performances (Shifaw et al., 2021; Van et al., 2020a). Clinical helminths infestations causes unthriftness, diarrhoea, innappetence and stuntedness among chickens (Phiri et al., 2007; Robel et al., 2003; Van et al., 2020b). *Syngamus trachea* causes respiratory distress. *Heterakis gallinarum* hosts *Histomonas meleagridis* which causes histomoniasis (typhllo-hepatitis) (Papini & Cacciuttolo, 2008). *Ascaridia galli* are very large nematodes that lead to intestinal obstructions especially in chicks. Helminths cause production losses and predispose chicken to microbial infections (da Silva et al. 2018). Helminths lower chicken vaccine responses and can affect the general immune system of chickens. However in mild and subclinical cases the signs of helminthiasis may not be observed (Ola-Fadunsin et al., 2019).

Anthelmintic resistance against conventional anthelmintics is an underestimated reality in chickens (Saemi Soudkolaei et al., 2021) yet no alternative anthelmintics are

provided. Phenothiazine and piperazine combinations which were effective on *Heterakis gallinarum*, *Ascaridia galli* and tapeworms were banned by the Food and Drug Agency (FDA) of the United states (Macklin & Hauck, 2019) . No comparable alternatives have ever been availed for the banned anthelmintics.

The purpose of this research was to document the ethnoveterinary practices in Soroti district, profile phytochemicals of selected plants, assess the efficacy and safety of the common plant extracts against chicken helminths.

The study was based on four theories of ethnomedicine studies; 1. The versatility, availability and diversification hypothesis which postulates that plants are used because they are accessible (Alencar et al., 2010; Bennett &Prance, 2000; de Albuquerque, 2006). 2. The theory of non-random plant selection which postulates that plants of related families tend to have linking chemical activity (Moerman, 1996) 3. Age, gender and dynamics of knowledge hypothesis which postulates that socio-cultural and demographic traits are related to people's plant knowledge (De Albuquerque et al., 2011; McCarter & Gavin, 2015; Souto & Ticktin, 2012) 4. Urbanisation & knowledge loss hypothesis that postulates that urbanisation decreases use of traditional knowledge (Brandt et al., 2013; Sogbohossou et al. 2015).

1.2 Statement of the Problem

Knowledge, attitudes and practices information regarding the use of ethnoveterinary materials in the control of poultry diseases in Uganda is very limited. There is loss of knowledge with time as communities may fail to pass-on ethnoveterinary knowledge (Aswani et al, 2018; Bussmann et al., 2018; Kumar et al., 2021). The ethnoveterinary practices in Soroti have never been explicitly documented. The scope of the available literature regarding ethnoveterinary practices in the control of chicken helminths is narrow and old (Aswani et al., 2018).

The particular active phyto-chemicals responsible for the action against the chicken helminths in Soroti haven't been determined despite the growing use of the ethnoveterinary practices.

Although ethnoveterinary practices are used in the control of chicken helminths in Soroti district; the efficacy and safety of these practices has never been scientifically tested. Efficacy and safety is a challenge in the use of ethnoveterinary medicines (Anderson, 2021; Li et al., 2020). The used materials may be ineffective or potentially unsafe to chicken and the consumers. Other researches of chicken ethnoveterinary practices in other places have inferences that were based on untested oral interview claims of chicken farmers (Kitata et al., 2017).

1.3 Objectives

1.3.1 General Objective

To evaluate the knowledge, attitudes and practices; phytochemical composition, efficacy and safety of selected medicinal plants used by poultry farmers in control of chicken helminths in Uganda: case of Soroti district.

1.3.2 Specific Objectives

i. To assess the ethnoveterinary knowledge, attitudes and practices for chicken helminth control in chicken farming communities of Soroti district.
ii. To analyse the phytochemical composition of the two most cited anthelmintic plant candidates.
iii. To determine the efficacy of crude extracts from selected plants used in control of helminths in chicken.
iv. To evaluate the haematological, biochemical and histopathological parameters of crude extracts from the two most cited anthelmintic plant candidates.

1.4 Research hypothesis

H_{01}: The quantity and type of phytochemicals from the selected plants used to control chicken helminths using acetone or ethanol solvents are not different from those commonly reported in literature as having actions against helminths.
H_{02}: The extracts from the selected plants are not able to cause worm immotility, deaths and damage of dermis of living Ascaridia galli; the extracts were not able to cause a faecal egg count reduction in naturally infected chicken compared to the therapeutic doses piperazine citrate and levamisole hydrochloride.

H_{03}: The extracts at a therapeutic dose can alter the haematological parameters, liver and kidney functions; and cause organ histopathology of the heart, kidneys and liver (not safe) compared to PBS, piperazine citrate and levamisole hydrochloride.

1.5 Justification for the study

Like most indigenous technical knowledge, ethnoveterinary knowledge though useful, are usually passed from generation to generation informally, undocumented and often secretively (Haque, 2019; Njeru, 2023). Thus, such wealth of information, practices and knowledge are often not documented, hence not available for wider usage (Anderson, 2021). Besides, there is often no verifiable evidence to prove that such practices are not only effective but also safe. Studies on efficacy and safety could provide empirical evidence on the usefulness of these products that could be readilyavailable cheaply for use in. This can reduce reliance on conventional drugs that are not only expensive but require technical veterinary support such as prescription or even administration. Conventional drugs also leave undesired residues in animal products (Patel et al., 2018).

Veterinary extension services are not always available or could be considered expensive yet ethnoveterinary products are usually free and accessible (Baker et al., 2018; Lem, 2019; Neal & Greenberg, 2022). There are no new conventional anthelmintics developed in the era of emerging anthelmintic resistance yet ethnoveterinary practices haven't been explicitly explored (Bhavsar et al., 2020). There is therefore a need to document the ethnoveterinary practices as alternative approaches prior to ascertaining their efficacies and safety for use.

1.6 Significance of the study

Documentation will increase the literature on common ethnoveterinary products and practices used in control of poultry diseases. This will be useful for dissemination of this indigenous knowledge for the benefits of a wider community. Soroti production department will take 50% of the patency rights. The animal health providers will use the inventories of this research to serve their clients in a farmer participatory approach. The natural product developers will have more opportunities of plant candidates against various diseases. This will lead to increased green synthesis and nano-technology in

chicken drug development. Ethnoveterinary products can be used in organic farming to provide consumers with safer chicken products.

The ministry of Agriculture, Animal Industry and Fisheries (MAAIF) can incorporate the information of this research in their farmer training manuals. Such enriched resources can lead to reduced losses in the poultry businesses. The information of the research can guide the regulation of use, supply and propagation of plant candidates. This also underscores the need to review the regulations on *Cannabis sativa* which is a restricted narcotic plant. The research ends the monopoly of specific herbalists and popularizes knowledge for all interested herbalists and users.

This work provides wide areas of farmer claims to be tested for efficacy and safety. This generates a wide scope plant extract purifying and fractionating for structural elucidation and drug development Generally; plant identification, phytochemical analysis and a possible structural elucidation is a key step to purification of the bioactive ingredients from the natural products in view of product development and commercialization (Hernández-Bolio et al., 2018).

1.7 Scope of the study

The study was done from 2020-2024 in Soroti district of Uganda, East Africa. It involved a survey where the participants and respondents were natives of Soroti district above 18 years and chicken owners. Only two solvents (ethanol and acetone) were used on each of the selected plants. Only fresh extracts were used in the proceeding experiments. For *in-vitro* assays, only very motile mature *A. galli* were used to study the effect of the extracts on the dermis and motility inhibition. *In-vivo* assays were done in the naturally infested indigenous chicken, only those which had FECG of above 200 were included in the experiment. Acute toxicity studies were done at the therapeutic dose of the extracts and control drugs.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Global outlook of the poultry industry

The global trade of poultry products has grown faster than of beef or pork yet the contribution from developing countries has remained insignificant (Daghir et al., 2021; Erdaw & Beyene, 2022). The demand for poultry products is increasing with the increasing population growth (Castro et al., 2023). Poultry have many advantages compared to livestock as human food (Vaarst et al. 2019). Poultry keeping is important for livelihood and improved nutrition for HIV/AIDS patients in the developing countries (Singh et al., 2022).

2.2 The chicken industry in Uganda

There were 3.2 million households that kept chicken in 2008 and 4.8 million households in 2021; the national chicken population was 37.4 million in 2008 and 57.8 million in 2021 (National Livestock census report, 2021). The chickens' populations didn't increase significantly possibly because sustainable disease control strategies were unknown or not accessible.

2.3 Poultry and its contribution to the livestock economy

Poultry production is 4.3% of the total value of agricultural production. 65000 metric tons of poultry meat and 0.9 billion eggs are produced per year (MAAIF, 2019). Uganda was estimated to have 37,443,880 chickens with Eastern Uganda having the highest number of 10,696,100 chickens (UBOS, 2008). The number of indigenous chicken in Uganda was 39,273,000 in the year 2017 and 35,415,000 in 2018 (UBOS, 2019). The number of other indigenous poultry (not chicken) in Uganda was 1,807,000 in the year 2008; 2,975,000 in 2017 and 2,273,000 in 2018 (UBOS, 2019). Chickens formed 60.7% of the poultry kept in Eastern Uganda in 2008 and the mean flock size was 11 chickens (UBOS, 2008). Chicken make 93% of poultry kept in Soroti district; the distribution of chicken in the sub-counties of Soroti district was as follows; Arapai sub-county (74,800), Gweri (66,091), Asuret (45,154), Tubur (28,347), Soroti (22,224), Katine (54,169), Kamuda (61,271), Northern Division (10,896), Eastern Division

(4,363), Western Division (7,686) (Soroti District Production Department, 2021). The chicken economy is plainly stagnant because of diseases and lack of effective drugs.

2.4 Chicken production systems in Uganda

2.4.1 Intensive production

These are production systems where more specialized breeds for particular purposes are reared by giving them much attention and requirements. It includes the deep litter systems, the slated floor systems and the battery cage systems (MAAIF, 2019). Biosecurity measures and compound feeds are provided. The housing is provided as semi-permanent or permanent structures. This production system is usually around peri-urban and urban areas. This system takes 25% of the production systems in Uganda (MAAIF, 2019).

2.4.2 Extensive Production

These are production systems that offer a wide expanse of land for the poultry, they include commercial extensive where the birds are reared in large numbers and are allowed to move either in gardens or on lawn. Traditional free range methods are also extensive systems where birds are allowed to scavenge on wide unlimited area (Jeni et al., 2021; Natukunda et al., 2011). Traditional free range is 55% of the production systems in Uganda. These systems predispose the birds to various parasites (Tamara et al., 2019).

2.4.3 Semi- intensive production

These are production systems where birds are allowed to wonder but with restrictions. The birds are few (less than 500) usually layers or broilers which are kept in temporary houses or other unconventional houses. The biosecurity system may be provided but it is not strict. In the run semi-intensive systems; the land is fenced and a house is provided therein. In the fold unit semi-intensive systems, the poultry house is small and portable for shielding birds on lawn or with feeds. This system takes 20% of the production systems in Uganda (MAAIF, 2019).

2.4.4 The backyard production

This is a semi-scavenging system where birds are kept within the small perimeter of the housing and provided with some feeds and water (Gentile et al., 2024). It is practiced by some urban farmers but the number of such farmers is not known.

2.4.5 Organic production

These are production systems intended to eliminate the use of chemicals in the control of parasites and diseases. Natural microbes, particular supplements and enzymes are used to control the poultry parasites (Abd El-Hack et al., 2022).

2.5 Free range poultry production in Uganda

This is the commonest production system in Uganda (MAAIF, 2019). It is a traditional approach where birds depend on hunting and scavenging in most cases with absolutely no supplementation of feeds. The birds usually have no conventional night shelters but share the human shelters or may roost on trees in the homestead. There are no restrictions of distance of movement, there are no diseases control measures and usually no biosecurity measures whatsoever. The system involves laying and hatching management interventions of designing nests for the birds and selecting the number of eggs that birds must incubate (Bonnefous et al., 2022). However, breeding is not monitored and the care of chicks happens naturally by the mother hen.

2.5.1 Major challenges to free-range poultry systems

Free ranging approaches cannot harmonise with biosecurity methods. The uncontrolled movements of birds predisposes them to various infectious diseases (Otte et al., 2021). There is mixing of various poultry species which have different susceptibilities to the different parasites and pathogens. There are increased risks of low egg hatchability and slow weight gain (Chaiban et al., 2020). The system exposes the birds especially the young ones to predation, accidents and deliberate killing by children (Zhang et al., 2023). There is uncontrolled breeding since there may be interacting of different neighbouring flocks. The birds are usually underfed if at all with an imagination that they have successful scavenging everyday which is usually not true.

2.5.2 Major diseases of free-range chickens

Diseases have delayed the transformation of the poultry sector to commercial farming (Aryemo et al., 2019). Diseases associated with poor management are more prevalent since health management is at low standards especially for the free ranging flocks (Otte et al., 2021). Diseases are more prevalent in particular seasons of the year with favouring weather conditions of temperature, humidity and air circulation (Byaruhanga et al., 2017).

2.6 Helminths parasites of chicken

Helminths are categorized into nematodes (round worms), trematodes (flukes) and the cestodes (tape worms). Nematodes are cylindrical, with a radial symmetry, a complete gastro-intestinal tract and sexual dimorphism. Trematodes are flat, unsegmented, with a bilateral symmetry with a blind ending gastro-intestinal tract of only the mouth. Cestodes are flat, segmented helminths with no gastro-intestinal tract. The table 3 below shows the common chicken helminths. Chicken nematodes are more common than trematodes and cestodes (Macklin & Hauck, 2019). Helminths causes various effects to the chicken depending on their lifecycle and predilection sites. Some helminths affect the gastro-intestinal tract resulting into poor digestion and reduced absorption of food (Yusuf et al., 2016).Worm migrations lead to mechanical damages of various organs. Helminths cause stress to chicken (Shohana et al., 2023). Tracheal nematodes obstruct the air passages and kill by asphyxiation (Macklin & Hauck, 2019). *Heterakis gallinarum* is an intermediate host for *Histomonas meleagridis* which affects the caecum. Simultaneous presence of *Heterakis gallinarum* and *Histomonas meleagridis* in chicken increases Th1 cells and decreases splenic CD4+ cells (Schwarz et al., 2011). *Ascaridia galli* develops various associations with bacteria resulting into more tissue damages and mortalities to chicken (Shohana et al., 2023). Helminths infections are highest in the traditional chicken farming systems (Macklin ; Hauck, 2019). The infections levels are influenced by seasonal changes which affect the helminths, the intermediate hosts or both (Macklin ; Hauck, 2019).

The common poultry nematodes are *Strongyloides avium, Trichostrongylus tenuis, Syngamus trachea, Hetarakis gallinarum, Heterakis isolonche, Heterakis dispar, Ascaridia galli, Eucoleus annulatus, Eucoleus contorta, Capillaria obsignata,*

Capillaria anatis, Capillaria caudinflata, Cheilospirura hamulosa, Gonngylonema ingluvicola, Tetrameres Americana and *Allodapa suctoria.* The common poultry cestodes are *Raillietina cesticillus, Raillientina echinobothrida, Raillientina tetragona, Choanotaenia infundibulum, Hymenolepis carioca, Hymenolepis cantaniana, Amoebotaenia cuneate, Metroliasthes lucida* and *Davainea proglottina.* The common poultry trematodes are *Zygocotyle lunata, Postharmostomum commutatum, Notocotylus imbricatus, Prosthogonimus anatinus, Echinostoma cinetorchis, Hypoderaeum conoideum and Echinoparyphium recurvatum* (Macklin, K.S; Hauck, 2019).

2.6.1 Identification of *Ascaridia galli*

Ascaridia galli are the commonest nematodes of chicken, they are yellowish white and stout with spicules of differing length. The males are usually shorter than the females. Males can be three to eight (3-8) centimetres long and 0.05-0.12 centimetres wide. They have a pre-anal sucker of approximately 220µm diameter and the tail end is rather flattened. The females are six to twelve (6-12) centimetres long and 0.09-0.18 centimetres wide and their tails tapers caudally (Faizullah et al., 2022). The females have a narrow cervical alae (Fioretti et al., 2016; Marchiondo et al., 2019). The eggs have a smooth and thick shell and are oviparous.

2.6.2 Life cycle of *Ascaridia galli*

Sexually mature worms harbour in the small intestines of chichens. Female worms lay eggs in the lumen of the small intestines and are passed out un-embryonated in faeces. The eggs take one to three weeks to develop the infective larval stage two (L_2) depending on the environmental temperature and humidity. Generally, eggs take one to four weeks to become infective after passing out in faeces. When the infective eggs are ingested, the L_2 hatches in the proventriculus or the duodenum. The larvae may enter the intestinal mucosa or remain in the lumen, however even they will return to the lumen to develop to adults. There may be erratic migrations in chicken to the ovary and eggs (Fioretti et al., 2016). The earthworms may be involved as transport hosts that carry the eggs to various points (Shohana et al., 2023). The prepatent period is approximately six weeks and it takes about four weeks for the hatched larvae to reach the adult stage (Marchiondo et al., 2019).

2.7 Diagnosis and control of poultry helminth diseases

2.7.1 Parasitological diagnosis

Involves procedures like direct slide smears for quick screening. In the Willis technique the faecal solution is made without addition of salts.

In the floatation techniques, salts are added to the water-faecal mixture to adjust the specific gravity of the solution. The object of the floatation methods is to harvest eggs on the top brim of the mixture. This technique is suitable for light eggs (nematode eggs). Sometimes centrifugation is incorporated in the floatation procedures (Sengupta et al., 2011).

Sedimentation is the method of natural settling of the suspension of faeces such that worm eggs are harvested in the sediment. The suspension is passed through a 1mm sieve and the filtrate is sedimented. This technique is suitable for heavy eggs (trematode eggs). Salts may be added and centrifugation procedures may be incorporated (Becker et al., 2016).

The Mcmaster egg counter is a glass slide of two layers separated by a known distance. The eggs per gram of faeces in the Mcmaster counting chamber can be counted. The Mcmaster methods may be improved by use of floatation solutions of high specific gravity (Pereckiene et al., 2007; Vadlejch et al., 2011). It is an accurate method if well used. Sweeny et al. (2011) found no significant accuracy differences between the Mcmaster technique and PCR methods for the strongylid nematodes. However, it should be noted that absence of eggs in the faecal samples does not always indicate absence of infection in the hosts.

2.7.2 Serological methods of helminths diagnosis

They have been used to detect various helminths (Roose et al., 2024). With this method; serum samples can be kept for long, large number of samples are handled and it's not labour intensive (French et al., 2016). However, the kits may be absent on the market and the antibodies are not species specific.

2.7.3 Molecular methods of Helminths Diagnosis

Species specific identification of helminths can be done using nucleotide primers. Sensitivity is increased when genus-specific primers are used and significantly decreased when species-specific primers are used (Dunn et al., 2020). It's apparently the most sensitive method but there may be lack of reference sequences and it's the most expensive method.

2.8 Control of chicken Helminths

Chicken helminths are controlled by routine management practices, use of allopathic drugs and in some cases ethnoveterinary practices. The management practices for the control of helminths require meticulous hygiene of water, feeds and equipments. Restricted movements, appropriate stocking density, grouping birds according to age and batch can also reduce helminths infestations. The synthetic drugs used to control helminths include albendazole, levamisole, piperazine, pyrantel, ivermectin, nitarsone, tetramisole, phenothiazine, methyridine, coumaphos, haloxon and thiabendazole (Macklin & Hauck, 2019). The EVP include use of certain plant products or any other non-conventional agents.

2.8.1 Challenges of synthetic antihelmintics

Synthetic anthelmintics are considered expensive by the users in the developing countries (Zirintunda et al., 2022). Synthetic anthelmintics are one of the residues in food animals (Adesiyun et al., 2021). The animal health services providers are usually not available to advise farmers on the choice and constitution of drugs, this makes farmers to guess resulting into various mistakes(Arvidsson et al., 2022). The drugs are not available in very remote settings of some chicken farming communities in Uganda (Jaime et al., 2022). Anthelmintic resistance has been reported in some communities and yet no new drugs have been developed. Some anthelmintics are not good enough to clear the parasites, they keep leaving a certain population of helminths that gradually develop resistance to the anthelmintics (Fissiha & Kinde, 2021a). Genetic modifications in the parasite occur following decades of application of synthetic anthelmintics. DNA replication errors subsequently promote evolutionary changes in the gene of the parasites to form stable DNA (Quinzo et al., 2022).

2.8.2 Ethnoveterinary approaches for chicken helminths control

Ethnoveterinary practices are considered when indigenous knowledge of nursing, training and healing animals (Rahman et al., 2023) and when these practices are readily available and acceptable (Oda et al., 2024). Alawa et al., (2002) and Alawa et al., (2010) mention that *Vernonia amygdalina* (Compositae) was effective against several helminths in calves, such plants are also potential candidates against poultry helminths. Githiori et al. (2005) in his *in vitro* work found that *Evodia rutaecarpa, Mentha cordifolia* and *Mangifera indica* were effective against helminths but this was not followed by trials in poultry. Adu et al. (2009) found *Carica papaya* to be effective against helminths in poultry but the details of the dose and regimen were not mentioned, *Carica papaya* was 13.9% efficacious (Chota et al., 2010), but the action against the various helminths was not determined.

Raza et al., (2016) mentioned *Anacardium occidentale, Allium sativum, Tribulus terrestris, Bassia latifolia, Piper betle, Morinda citrifolia, Cassia occidentalis and Aloe secundiflora* to be effective against helminths but did not determine the active compounds and did not state the regimen. Siamba et al., (2007) determined that *Tephrosia vogelli* and *Vernonia amygdalina* leaf extracts were effective against helminths but they used a narrow perspective of one helminth, there is need to determine the effect of the extract on other poultry helminths other than *Ascaridia galli*. Pande et al. (2007) mentions the general families of Asteraceae and Fabaceae as effective, *Azadirachta indica* was mentioned to be particularly effective against *Ascaridia galli.*

Mwale & Masika, (2009) mention Aloe *ferox, Helichrysum splendidum, Agave sisalana* and *Millettia grandis* as having antihelmintic potential; Mwale et al., (2015) determined the *in vitro* effectiveness of *Aloe ferox, Agave sisalana* and *Gunnera perpensa* against *Heterekis gallinarum*, the success was not proven against other helminths and no poultry *in vivo* trials were done. Tandon et al., (2011) found extracts of *Trifolium repens* (Fabaceae), *Flemingia vestita* (Fabaceae), leaves of *Psidium guajava, Houttuynia cordata,* stalk of *Clerodendrum colebrookianum, Lasia spinosa* and *Centalla asiatica* effective against earth worms and helminths in laboratory animals but did not determine the active compounds or follow up with poultry trials. Use of EVP reduces the overrelying on antimicrobials which are implicated in AMR

development (Feiyang et al. 2021; Pokharel et al. 2020). Over use of antimicrobials has been reported among commercial poultry farmers (Majalija et al. 2023).

2.8.3 Challenges of Ethnoveterinary approaches to helminths control

Usually one herb may be claimed to be effective against a variety of diseases (Chaughule & Barve, 2024; Dhama et al., 2015). The particular plants of interest are not freely available in all regions. Some important plants are getting extinct because of overuse and human encroachments on the natural conservation areas (Geldmann et al., 2019).The concentrations of the plant metabolomes vary with the variations of the soils on which the plant grows. The plants accumulate toxic materials especially in areas of high environmental toxicants (Ssempijja et al., 2020). Many ethnomedicinal plants are contaminated with unacceptable levels of heavy metals (Kasozi et al., 2021). However EVP remains with many controversies, obscurities (Ahmed & Ansar, 2016) and most claims haven't been validated. *Carica papaya* L. extracts have been associated with gonadal effects and sex reversal that leads to infertility in fish (Iipinge et al., 2023; Radwan et al., 2023), however related challenges haven't been studied in chickens. Capsicum annuum has strong pungency properties (Das et al., 2023) although the magnitude of this challenge in chickens is not explicit in literature.

2.9 Extraction of ethnoveterinary plant compounds

2.9.1 Soxhlet extraction

This uses a continuous flow of solvent alongside digestion of the plant material to yield the extract (Arceusz et al., 2013). It's an old method which is still relevant for extraction. The sample in a thimber-holder is slowly filled with condensed pure solvent from a distillation flask (de Castro; Ayuso, 2000). Soxhlet extraction yields a lower volume of the extract and there are possible degradations during the process (Zhang et al., 2018). Though considered a benchmark method, other methods may be preferred (Nguyen et al., 2025). It's a time consuming procedure and much solvent of a superior quality is required (Trolles-Cavalcante et al., 2021).

2.9.2 Ultrasonic assisted extraction (UAE)

This uses the principle of ultrasonic vibration in the direction of the extracted sample in the presence of a solvent (Vinatoru et al., 2017). Because of the mechanical effect

caused, there is a better penetration of the solvent into the plant material (Pan et al., 2012).The intended purpose of the extract dictates the solvent used. Extracts for use in the gastro-intestinal tract should be made using alimentary grade solvents (Vinatoru et al., 2017). It's fast, simple, uses less solvent and several samples can be extracted simultaneously. The quality and quantity of the extract is influenced by the sample particle size, PH, temperature and the amplitude of sonification (Arceusz et al., 2013).

2.9.3 Supercritical fluid extraction (SPE)

This is a specialized method done after HPLC. The method uses supercritical fluids like carbon dioxide which acts as a non-polar solvent (Sapkale et al., 2010). Extracts can be produced with no solvent residues if no entrainers are used (Dong, Dai, & Lei, 2018). It doesn't affect thermally labile compounds and uses a small volume of solvent. Carbon dioxide is not flammable, it's inert, cheap and odourless which is an advantage in the nutraceutical setups (Leo et al., 2005). It takes a very short time but the yield is also affected by the form of the plant material, its homogeneity, rate of flow of solvent, temperature, time and pressure.

2.9.4 Accelerated Solvent Extraction (ASE)

This method uses very high pressure and temperature. Organic solvents can be used on solid samples that remains in the cell during the extraction (Richter et al., 1996). The method allows easy sample preparation but it makes a non-selective extraction and the apparatus is very expensive (Giergielewicz-Mozajska et al., 2001).The volume of the extract depends on the duration of the procedure (Schäfer, 1998) and the polarity of the used solvents (Herrero et al., 2005). It can be used to extract even high humidity samples. It takes a short time with better penetration of the solvent and the method uses a small volume of the solvent of 50-100ml (Richter et al., 1996).

2.9.5 Shake extraction

This method uses the principle of increasing the surface area where the sample interfaces with the solvent. The sample is placed in the solvent and shaken at a particular speed. Rotary mixers were found to be more yielding than orbital platforms (Juanola et al., 2002). Shake flask extraction method is as efficient as the soxhlet extraction method (Juanola et al., 2002).

2.10 Phytochemical analysis of plant extracts

Phytochemicals can be primary or secondary (Kalaiselvi et al., 2016). Primary phytochemicals include chlorophyll, common sugars and proteins while secondary phytochemicals include terpenoids, alkaloids and phenolics (Krishnaiah et al., 2007). Proteins can be quantified using the micro Kjeldahl's method and vitamins using UV/visible spectrophotometer (Hussain et al., 2011). Secondary phytochemicals can be qualitatively analysed using the procedures of Parekh & Chanda (2007). Quantitative analysis of phenolics is done using the colorimetric methods (Haq et al., 2012). Flavonoids can be analysed using the methods of Jun et al., (2002). Generally secondary phytochemicals can be qualitatively analysed using the methods of Sofowora (1993) and Harborne (1998).

2.11 Gas Chromatography-Mass Spectrometry (GC-MS)

It's a technique that does qualitative and quantitative analysis of chemical compounds, the gas chromatographer separates a vaporised sample's components (Ukwubile et al., 2019). The compounds ought to be volatile to travel through the GC-column. As the vapour is pushed up, different molecules cling to the column coating for different time periods and exit one at a time.

Compounds from the GC are pushed to the MS which indentifies and quantifies sample components according to their mass to charge ratios. In the MS, ionisation takes place by blasting molecules with high energy electrons to make charged molecule fragments. The charged fragments are pulled through a magnetic-electric field according to their mass to charge ratio. The detection is because of hiting the detector at each mass to charge value. The data is matched to reference libraries to determine the type of separated compounds.

2.12 Phytochemical action against helminths

Condensed Tannins cause paralysis and death of helminths (Greiffer et al., 2022). Flavonoids affect the calcium pump and ATPase leading to the death of the helminth (Adak & Kumar, 2022). Phenolic acids affect cell signaling pathways and gene expressions leading to the death of the helminth (Wei & Tanokura, 2015). Benzo (c) phenanthridine or isoquinoline alkaloids damage helminth neurons leading to the death

of the helminth (Verpoorte, 2005). Saponins affect mitochondrial action and also alter the permeability of the cell membrane leading to death of the helminth (Morgan & Wilson, 1999). Yeast encapsulated terpenes inhibit neurotransmission and lead to helminth paralysis, they also inhibit hatching of helminths eggs (Sponsel, 2003). BITC causes helminth DNA and cuticle damage (Akram et al., 2021). Isoflavones cause helminth paralysis and inhibit energy utilization (Hampl et al., 2009). Artemisinin and its derivatives inhibit neurotransmission resulting into worm paralysis and can affect mitochondrial action resulting into worm death (Zeyuan et al. 2018).

2.13 Toxicity of ethnoveterinary compounds

All chemicals and natural products could induce toxicities especially when administered in very high doses (Abdel-Daim et al., 2018). Most of the plants used (81%) for treating animals have toxic effects unless dosed prudently (Abdisa & Dilbato, 2024). Although many plants have been assessed to have ethnoveterinary potential, not many have been assessed for toxicity (Sunder, 2016). The grades of the toxicities may vary for similar plants because of the possible different levels of soil contaminations with minerals (Gworek et al., 2021). Most of the studies have determined cytotoxicity on the parasitic agents but simulations of host living cells were not done. Mathew et al., (2014) determined cytotoxicity against micro-organisms but not do simulations of cytotoxicity oncells of the hosts. McGaw et al., (2007) found 30% of the plants extracts used in South Africa to have toxic effects against brine shrimp larvae of *Caenorhabditis elegans* but host trials were not done. *Vernomia amygdalina* though medical but has toxic effects although Yeap et al., (2013) asserts no toxicity for the plant in mice up to 5000mg$(kg)^{-1}$ body weight. *Aloe vera* leaf extracts were toxic to chicks and led to death in 20% (2/10) of the chicks and a rise in white blood cell counts at a dose of 640mg$(kg)^{-1}$ (Nghonjuyi, 2015; Nghonjuyi et al., 2016), but it's still used. *Acacia nilotica, Tetradenia riparia, Aloe arborescens* and *Crassula multicava* though still used in poultry but were reported as toxic (Sserunkuma et al., 2017).

Thymol oil from *Thymus vulgaris* (lamiaceae) used in poultry to lower pathogen concentration had *in vitro* cytotoxic activity of an unknown mechanism (Ezzat Abd El-Hack et al., 2016). *Cremaspora triflora, Measa lanceolata* and *Hypericum roeperianum* were reported as cytotoxic (Elisha et al., 2017) but cytotoxicity on host cell simulations was not done. Bizimenyera et al, (2008) declared *Peltophorum africanum* sond

(Fabaceae) nontoxic to host cells but the selective index wasn't determined. *Vernonia guineensis* didn't produce toxic effects in rats even at a maximum concentration of 4000mg $(kg)^{-1}$ (Toyang et al., 2012) but detailed organ effects and haematological effects were not outlined. Agaie et al., (2007) found the aqueous leaf extract of *Anogeissus leiocarpus* fairly not toxic in rats with no death even at a dose of 3200mg $(Kg)^{-1}$ but found dose dependent signs of toxicity. *Carica papaya* L. extracts have been showed to have no effects rats (Taychaworaditsakul et al., 2024) but related studies haven't been done in chicken. *Capsicum annuum* L. fruit extracts are said to cause effects in humans especially when given in excesses (Johnson, 2007; Lučić et al., 2022), however no related studies have been conducted in chickens.

2.13.1 Acute toxicity

Acute toxicity occurs just after administration or in within 24 hours after administration of a substance (Chinedu et al., 2013). It can be assessed using the following ways

2.13.1.1 Cell line models

Cell line Resazurin reduction assay (Berg et al., 2015) which assesses V79 cell vitality and use of heatmaps (Transcriptome profiles) (Louisse et al., 2019) which assesses cell viability.

2.13.1.2 Use of plant models

Lettuce (*Lactuca sativa*)-seed germination or root elongation protocols are used. The test material is put on filter paper and delivered by water (Rafique et al., 2025). An inert matrix is used because seed germination and root elongation do not occur in soil matrix. The plants are left to grow for 14 days and the EC50 determined using indicators of germination, seedling survival and general plant health. Green algae (*Selenastrum capricornutum*).

2.13.1.3 Use of animal models

In the animal models the test sample is administered and monitoring is done (Erhirhie et al., 2018). Lethality assays of Fresh water rotifer (*Brachionus calyciflorus*) (Pawlak et al., 2022) and Brine Shrimp (*Artemia salina*) (Romero-Chávez et al., 2024) are also toxicity study models. Water flea test (*Daphnia magna*) which assesses levels of immobilization of the fleas by the substance under test (Albert & Bloem, 2023). Fathead

minnows test (*Pimephales promelas*) which assesses changes in behaviour and morphology of the minnows (Vignet & Parrott, 2017). Mysid shrimp test (*Mysidopsis bahia*) which assesses the feeding ability and lethality of the shrimps (Engström et al., 2001). Earthworm test (*Lumbricus terrestris*) which measures tests lethality as the end point (Elliston & Oliver, 2020). Microtoxi test (*Photobacterium phosphoreum*) which measures lethality of the luminescent bacteria (J. Li et al., 2021).

2.13.2 Protocols for acute toxicity studies using laboratory animals

2.13.2.1 Lorke's method of determination of acute toxicity

Nine animals are used in phase one in groups of three per group at a rate of 10, 100 and 1000mg/Kg body weight of the substance. In case no mortalities are observed then phase two is done. Phase two has three animals which receive 1600, 2900 and 5000mg/Kg body weight of the substance.

$$LD50 = \sqrt{(D0XD100)}$$

Where LD_{50} is the dose that kills half the number of the study subjects, D_0 is the highest dose that gives no mortality and D_{100} is the lowest dose that produces mortality (Lorke, 1983).

2.13.2.2 Karber's method of determination of acute toxicity

Various groups of animals are used each of five animals. The first group is administered with only the vehicle material for the test substance like water or DMSO. The second group is administered with the lowest concentration and the other groups are administered with an increasing concentration of the test substance.

$$LD50 = LD100 - \sum(\frac{aXb}{n})$$

Where LD_{100} is the least required to all the experimental animals, *a* is the dose difference, *b* is the mean mortality and *n* is the group population (Kärber, 1931)

2.13.2.3 Up and down method of determination of acute toxicity

One animal is usually used and the effects of the doses are monitored sequentially. One animal is dosed and monitored for 48 hours for any adverse effects or mortality. The

doses of the substance is either increased or decreased depending on the outcome of the current experiment. The experiment is ended if LD_{50} is established or when the highest dose (2000-5000) mg/Kg body weight is reached without mortality, in such a case we report that LD_{50} is greater than 5000mg/ Kg.

2.13.2.4 Determination of acute toxicity (Chinedu et al., 2013)

This method has three stages; the first stage has four animals in four groups. Animals are administered with different doses of the test substance. Animals are observed for the first one hour and then 10min in two-hour intervals for 24 hours. In case of no mortality stage two is started. Stage two has three animals and three groups which receive higher doses than stage one. Monitoring is done for the 1^{st} one hour and 10minutes in 2hour intervals for 24 hours. In case of no mortality stage three is started. Stage three has three animals in three groups which receive various doses with 5000mg/Kg body weight as the highest. If mortality is not observed then LD_{50} is greater than 5000mg Kg body weight of the substance. The LD_{50} is confirmed using two animals.

2.14 Organ histopathology

Diseases and toxins cause various changes in the liver for example sinusoidal congestion, fatty degeneration, necrosis in hepatocytes, central vein dilation, increase in apoptotic cells and fibrosis (Loghman et al. 2014). Other liver histopathological changes include hepatocellular vacuolation, hepatic haemorrhages, intraparenchymal bleeding and showing of collagen with changes in the vascular walls (Trott et al., 2014). *Curcuma longa* L. was reported to cause infiltration of mononuclear cells proliferation of biliary epithelium and periportal hepatocyte degeneration (AL-Sultan & Gameel, 2004). *Carica papaya* Linn distilled water extract is said to have reduced oxidative stress and liver cell injury induced by carbon tetrachloride in rats (Shaban et al., 2021).. *Carica papaya* L.extracts are effective against non-alcoholic fatty liver disease which is associated with oxidative stress (Deenin et al. 2021), they are able to restore altered hepatic tissues in type 2-diabete mellitus rats (Roy et al., 2023). *Capsicum annuum* L. is said to be hepatoprotective because of high levels of capsaicin (Li et al., 2024), however there are evidences of liver damage if high doses of immature pepper extracts are used (Morittu et al., 2020). The mentioned experiments above were not replicated

in chickens. Toxic materials causes the following changes in the kidney; aflatoxins causes changes in glomerular diameter, glomerular basal membrane thickening, degeneration of proximal tubules and cortical fibrosis (AL-Sultan & Gameel, 2004). Colibacillosis causes glomerular degeneration, congestion and infiltration by inflammatory cells. Colibacillosis also causes cell damage and necrosis (Andayani et al. 2018). *Carica papaya* L. was reported to be nephroprotective in rats (Chandra et al., 2022), however it may lead to acute kidney injury under some conditions (Raja et al., 2019). *Capsicum annuum* L. has been reported to be nephroprotective (Musolino et al., 2024). No related studies have been done in chickens.

Histopathological changes of the chickens heart includes pericardial effusion, thickened pericardium, fibrous visceral pericardium, adhesions of parietal and visceral parts of pericardium (Olkowski et al. 2003). Other changes can be haemorrhages in epicardium and myocardium with zones of myocardial infarctions. *Carica papaya* L leaf extracts have antioxidant properties and were found to be cardioprotective in rats (Ademuyiwa et al., 2023). *Capsicum annuum* L fruit extracts have been reported to be cardioprotective (Shirani et al., 2021), however the detailed effects on the cardiac muscles was not assessed. The mentioned experiments haven't been replicated in chickens.

2.15 Haematological indicators of toxicity in poultry

Stress factors decreased concentration of red blood cells and increased the haemoglobin concentration (Gorelik et al. 2020). High concentration of aflatoxins leads to leucopenia, lymphocytopenia and heteropenia (Omnia et al. 2020). However, literature on the effect of use of ethnoveterinary practices on poultry haematology is not widely documented yet the practice is popular.

2.16 Organ Function tests of toxicity

2.16.1 Liver toxicity indicators

Liver damage due to toxic materials and diseases can be assessed by liver histopathology (Larrey et al., 2024). The liver histopathological changes indicative of toxicity includes one or more of the following conditions; hepatic vacuolization, degeneration, inflammations, heterogeneity and necrosis. Changes in the levels of liver

serum enzymes is also used as an indicator of liver damage or toxicity (Orisakwe et al., 2018). The liver serum enzymes assessed for liver damage are Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Glutamic Oxaloacetic Transaminase (GOT), Glutamic pyruvic Transaminase (GPT) and Alkaline Phosphatase (APH). *Carica Papaya* L. leaf extracts are said to have caused no effect on liver enzymes of *Clarias gariepinus* juveniles (Oparaku et al., 2024). *Capsicum annuum* L. lowered the level of liver enzymes in rats that had induced inflammation and apoptosis (M. Das et al., 2018). The experiments were not replicated in chickens.

2.16.2 Renal toxicity indicators

Damage on the kidneys is showed by changes in the serum concentrations of Blood Urea Nitrogen (BUN), creatinine, uric acid and changes in serum osmolality (Gounden et al., 2021). However, in laboratory animals, changes kidney weight and histopathological changes are also plausible indicators of nephrotoxicity.

2.17 Conclusion on the literature reviewed

Chickens take the biggest percentage of poultry in Uganda. Local breeds are over 70% of all the chickens owned in Uganda. Chickens are owned by almost all rural and semi-urban households but their populations and contribution to the GDP is still meagre because of parasites and diseases. In many cases synthetic pharmaceutical substances are either not accessible, not effective or prohibitively expensive.

The free-range production system takes the biggest percentage of the production systems in Uganda. The system makes the chickens vulnerable to parasites and other infectious agents because it is done with absolutely no biosecurity measures. The intensive systems are mainly in the urban areas but also usually lack the required management standards and biosecurity.

Helminths parasites are the commonest parasites of chickens and they affect the way the parasites respond to vaccinations. Helminths predispose to other infections by affecting the immune system. The direct and indirect chicken production losses due to helminths can’t be easily quantified. The control of chicken helminths depends on proper management, use of conventional drugs and lately ethnoveterinary practices. The EVP have become more common because of challenges of affording or accessing

the conventional drugs. Most EVPs are acceptable in organic farming. EVP is also an alternative to banned drugs and ineffective conventional anthelmintics. The EVP have limitations because not all have been proven for safety and efficacy. The available EVP information is usually inconsistent yet mingled with obscurities and fallacies. The regimen, indications and contraindications of EVP are not explicit as one material may be linked to very many actions. The scope of most literature was majorly claiming missing empirical proof of *in-vitro* and *in-vivo* assays in chickens.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Study Design

Sequential mixed design was used starting with qualitative approach before progressing to quantitative approaches. The qualitative approach was in form of modified ethnography with methods of FGDs and series of chicken farmer engagements (grounded theory) for objective one. Quantitative methods were used for objective 2, 3 and 4. The study employed mixed methods in data collection. Initially a cross-sectional survey was conducted to identify ethnoveterinary practices, knowledge and attitudes of chicken farmers in Soroti district. The analytical methods were used to identify the phyto-active compounds in the most mentioned plants. Subsequently, *in vitro* and *in vivo* experiments were done to test the efficacy and safety of crude extracts from selected plants sampled during the survey.

3.1.1 Research approaches in ethnoveterinary survey

After the ethical clearance at the various levels, the specific sub-counties were agreed on using the Soroti district production data. The participants and respondents signed a consent form to participate in the research. The underpinning theories were stated to guide the details of the research approaches. Qualitative and quantitative approaches of data collection were used. The qualitative approaches involved focus group discussions, observations, semi-structured questionnaires and structured questionnaires. The information was recorded as audios, videos and photographs. Quantitative data drawn from the questionnaires and used to develop ethnomedicinal indices of frequency of citation, percentage respondent knowledge.

3.2 Study area

The study area was Soroti district of Uganda. (Figure 1). Soroti district is located at Latitude 1º42ʾ47.4516ʾʾN and Longitude 33º36ʾ22.986ʾʾE. It is one of the areas with high number of households keeping chicken (UBOS, 2024). Soroti district is generally a flat land. It has various vegetations like wooded savannah, grass savannah riparian vegetation, forests and vast wetland vegetations (Soroti District Local Government, 2018). It receives rain around April-June and August-October with most of the year

being very dry. It has an average minimum temperature of 18^{o}C and an average maximum temperature of 31.3^{o}C.

3.2.1 Climatic conditions of Soroti district

Soroti district is located at Latitude 1o 42’ 47.4516’’N and Longitude 33o36’ 22.986’’ E. In May 2024 the temperature ranges were 18.9-27.1o C with average humidity of 84%. The rainfall of 192 mm (7.56’’) precipitation and the UV index range of 3-5 mW/m^2.

3.2.2 Study populations

Soroti district has a population of 296,833 people with a population density of 248.9 persons per square kilometre (UBOS, 2024). Most of the people (80%) are small holder farmers, their livelihood is dependent on various livestock and poultry (Friis-Hansen, 2008).

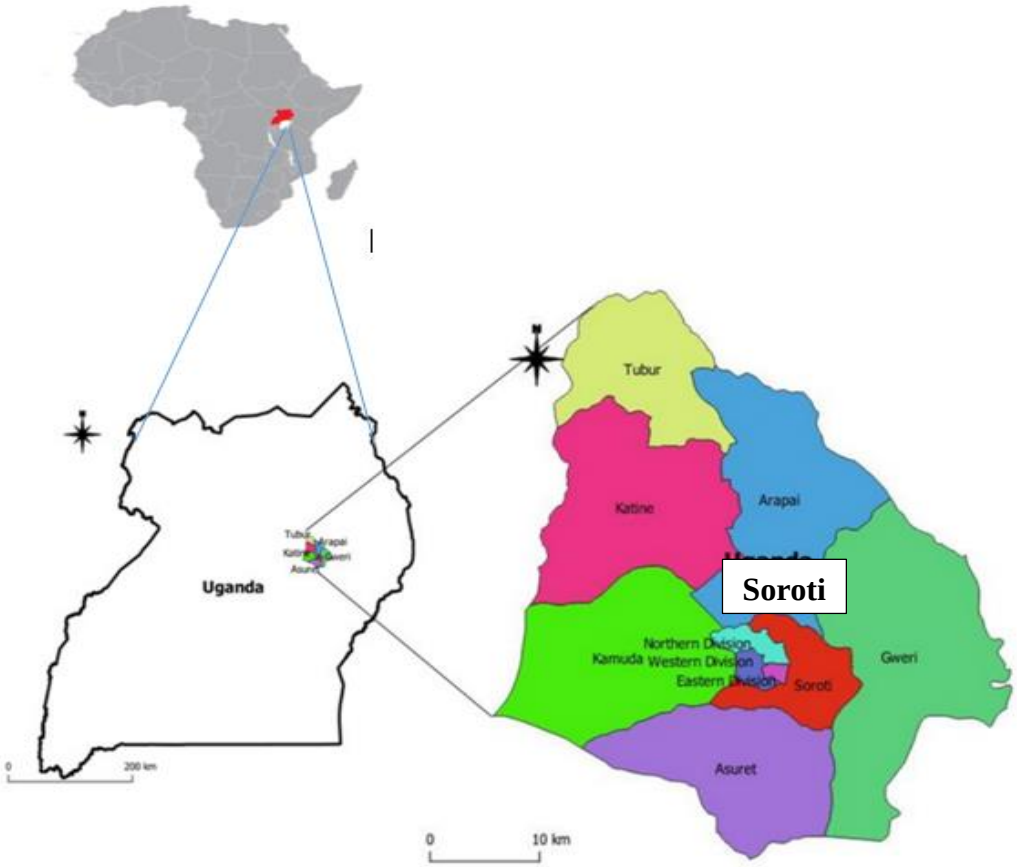

Figure 1: Map showing the sub-counties of Soroti district of Uganda

3.3 Description of the study components

a) Human subjects: These were met in a survey during the Farmer questionnaires, KII and FGDs. Farmers were adults that keep chicken or anyone delegated to take care of chicken at the farmers' households. Farmers having the highest number of chicken were selected for the interviews and invitation for the FGDs. The Key informants were veterinarian, veterinary para-professionals and researchers in Soroti district.

b) Plant samples: These are the materials that were collected during the survey. These were collected during and after interactions with respondents. The village names and GPS coordinates were recorded and also pinned on the plant materials. Expected materials were plant parts from different families. The samples were air dried pending ranking and submission for taxonomic identification and assigning voucher numbers.

c) Chicken: Indigenous chicken were procured from any reputable hatchery in Uganda.

3.4 Sampling

3.4.1 Objective one (Documenting current ethnoveterinary knowledge, attitudes and practices)

For making inventory of ethnoveterinary knowledge for chicken disease control and establishing practices for chicken helminth control; FGDs were conducted up to saturation. Seventy-eight purposively selected chicken owners with over 50 chickens according to the sub-county production records participated in the detailed questionnaires.

Documenting the implication of knowledge, attitudes and practices of ethnoveterinary disease control (quantifying the KAPs) required sample size determination as follows;

$$n = z * z * \frac{Pexp(1 - Pexp)}{d * d}$$

Where n is the sample size, z is the constant at 95% confidence level, P_{exp} is the estimated percentage of chicken farmers that use ethnoveterinary drugs and d is the desired precision.

z = 1.96, d= 0.05 and P_{exp} =0.66 (Natukunda et al., 2011; Thrusfield, 2007).

Hence a sample size of 407 respondents were selected from the population for questionnaires.

3.4.2 Sampling Techniques

Soroti district was selected because it has a high number of households that keep chicken and it is a commercial centre for local chicken. Four rural sub-counties with the highest number of households with chicken (Arapai, Katine, Gweri and Kamuda) were selected. Two of the urban sub-counties with the highest number of households with chicken (Northern Division and Western Division) were also selected. Sampling frames of lists of chicken farmers were developed from the sub county production records. Participants were required for FGDs and respondents for farmer questionnaires per sub-county. Chicken owners were selected randomly (simple random sampling) per parish from any two parishes with the highest number of chickens to participate in the FGDs per parish. Parishes that participated in the FGDs were (Dakabela & Arabaka) for Arapai sub-county, (Ojama& Oculoi) for Katine, (Gweri & Dokolo) for Gweri, (Aminit & Olio-Awasi) for Katine sub-county, (Opiyai & Otucopi) for Northern, (Oderai &Majengo) for Western division. Other chicken owners were randomly selected for individual questionnaires from the parishes that had not participated in FGDs.

3.5 Data Collection

3.5.1 Documenting current ethnoveterinary knowledge, attitudes and practices

This was achieved using the approaches of the grounded theory. It involved the use of social processes to unveil EVM phenomenons (Noble & Mitchell, 2016). A modified ethnography approach was followed using FGDs and Farmer interviews methods with open ended guiding tools of a qualitative study design (Tresca et al., 2020). This was started by making an inventory of ethnoveterinary Knowledge for chicken disease control and documenting ethnoveterinary practices for chicken helminths control which involved;

i. Traditional focus group discussions of not less than eight adults (above eighteen years) per group were conducted at each of the sub-counties; The groups were organised to allow the participants to discuss freely (Fern, 1982; Kitzinger,

1995). Twelve non homogenous FGDs were performed, two FGDs were done for each of the sub-counties to reach saturation. Arapai- Dakabela (nine males and nine females of an average age of 35 years), Arapai-Arabaka (ten males and four females of an average age of 25 years), Gweri-Gweri (ten males and three females of average 35 years), Gweri-Dokolo (ten males and one females of average 40 years), Kamuda-Aminit (ten males and five females of average 40 years), Kamuda-Olio (six males and seven females of average twenty years), Katine-Ojama (twelve males and three females of average 35 years), Katine - Oculoi (seven males and three females of average 30 years), Northern Division-Opiyai(seven males and four females of average 45 years), Northern Division-Otucopi (six males and five females of average thirty years), Western Division-Oderai (three males and 10 females of average 40 years), Western Division-Majengo (nine males and five females of average 35 years). All involved signed to consent willingness to participate in data collection. Voice recorders were used to record the discussions with the consent of the farmers. The participants usually walked the research team around, showed the mentioned plants and made illustrations of the preparations. FGDs helped the researcher to understand the knowledge and practices to inform better experimental approaches to do the study.

ii. Farmer interviews at the farmers' premises with 78 chicken owners; plants, plant parts, local names, methods of preparation and use were recorded. The common undesired effects from the plant products were also recorded. The plants and plant parts were ranked according to the number of mentioning by the various chicken owners.

iii. Collection of samples of plant materials for taxonomic identification and assigning of voucher numbers; the objective was concluded by studying the implications of knowledge, attitudes and practises of Ethnoveterinary control of chicken diseases using KAPs scaled chicken owners'questionnaires administered at the premises of 407 respondents.

3.5.1.1 Conceptual framework for documenting the ethnoveterinary knowledge, attitudes and practices for chicken disease control

Our study was based on the assumption(s) that chicken owners use ethnoveterinary alternative medicines. Such alternative interventions are treatment ideas that are passed from one generation to another by word of mouth. The choice of this alternative is common with small holder chicken owners or where flock sizes are low as in most sub-Saharan households (Gobvu et al., 2023). The drivers to the use of EVM may be inadequate veterinary extension services, expensive synthetic drugs, ineffective synthetic drugs, need for organic foods and traditional inclination (Bamidele et al., 2022). However, the policies in most African countries using ethnoveterinary alternatives have not been streamlined regarding use, copy rights, packaging and scaling up for marketing (Zirintunda et al.2022). Because EVM are usually accessible and acceptable as alternative chicken medicines, they can be harnessed to change lives especially in developing countries as in line with sustainable development goal one and two.

Ethnoveterinary medicine is simulated to the Indian Ayurveda, Traditional Chinese Medicine which is widely practiced in those culture setups (Mahapatra, 2019). This practice is after traditions, prolonged observation and beliefs. It's not based on the modern theories of medicine approval and use. The proof is only based on the end result especially by repeated use for generations. The traditional medicine users are thought to have a wide scope of knowledge regarding treating, ethnobotany and nature of species being treated but this is not based on structured scientific reasoning (Schillhorn, 1997). EVP is used in parasitology, pathology, pharmacology and physiology with inputs from various biological sciences (McCorkle, 1986). It was also considered that progress of ethnomedicine requires inclusive stakeholder engagements to bridge the gaps in the knowledge systems through participatory research (Reyes-Garcia, 2023). However, recent studies have underemphasized the development and connections of ethnomedicine identification and use (Gaoue et al. 2017).

Kutesa (2018) noted that; people are in continuous search for knowledge in the formal and informal realms. People in the various parts of the world have used plants for treatment of various diseases since pre-historic times (McCorkle, 1986). However, there is limited epistemic validation of herbal medicines and limited convincing epistemological guidelines. The knowledge of ethno-medicine is considered true

because it's related to the metaphysics of facts, it's therefore nearly in tandem with the Bayesian theorem. This analysis assessed how chicken owners frame ethnoveterinary aspects to answer their specific production problems. This was specifically to explore the knowledge, attitudes and Practices of chicken owners and their implications. In this case, 'knowledge" is the sum total of the actions of the chicken owner to treat chicken diseases. "Attitudes" are the preferred ways of behaving towards the practice of ethnoveterinary medicine. "Practice" means the way of life and actions tailored to chicken diseases control. Knowledge was composed of knowing common plants for use, their preparation, doses, and methods of administration, adverse effects and plausible responses to adverse effects. Positive attitudes were composed of being convinced of the plant efficacy, accepting the need for scaling up of plant drugs, accepting the need for preservation of medicinal trees, considering the plant drugs as safe and accepting the mythology associated thereof. Practices included those of planting medicinal trees, sharing medicinal knowledge, keeping records, hygiene during medicinal preparation, adding of materials like alcohol or honey, use of human drugs alongside and performing of rituals during use. Age, gender, education and place of residence were the assumed predictors which we used to develop the linkage of knowledge, attitudes and practices. The study traces the feasibility of use of plant medicines in chicken. The study is justified by the urgent need of effective alternative medicines to circumvent the prevailing challenges of anti-microbial resistance and abject lack of drugs in some low developing countries.

The areas where FGDs and farmer interviews took place are shown in **Figure 2**.

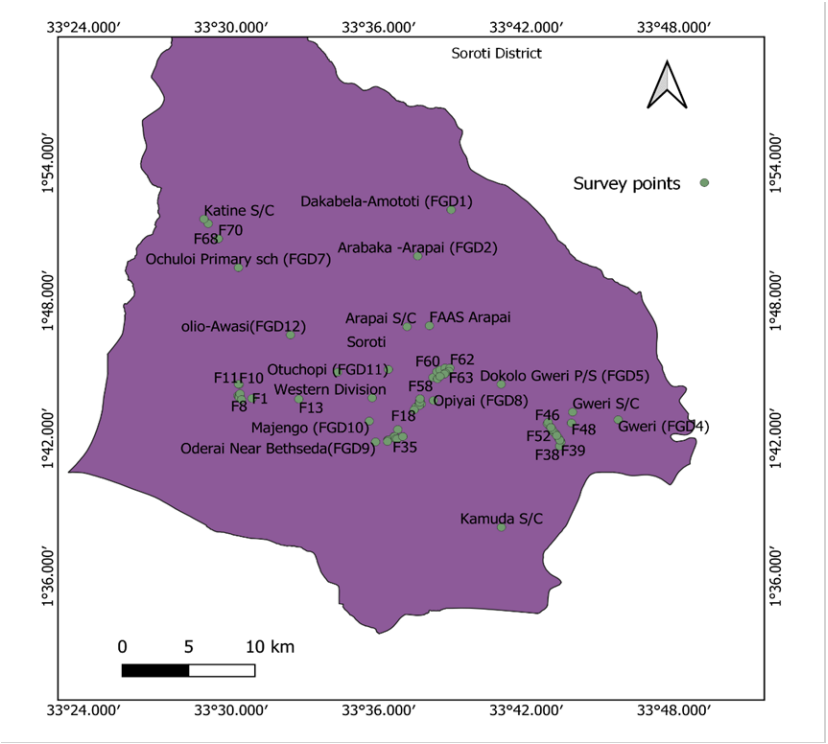

Figure 2: Map showing the survey points in Soroti district

3.5.1.2 Methodology for studying ethnoveterinary practices for chicken helminths control

Decribed in section 3.5.1 above

Frequency of citation **(FC%) =**

$$\frac{\boldsymbol{Number\ of\ mentions\ for\ a\ particular\ species}}{\boldsymbol{Total\ number\ of\ mentions\ for\ all\ species}} \boldsymbol{X\ 100}$$

Percentage of Respondent Knowledge **(PRK%) =**

$$\frac{\boldsymbol{Number\ of\ people\ interviewed\ citing\ the\ species}}{(\boldsymbol{Total\ number\ of\ people\ interviewed})} \boldsymbol{X100}$$

3.5.1.3 Informant consensus factor (ICF)

$$\textbf{ICF=}\ \frac{\boldsymbol{Nur-Nt}}{(\boldsymbol{Nur+1})}$$

Where N_{ur} is the number of times informants mentioned using plants against chicken helminths and N_t is the number of different plant species cited against chicken

helminths. An ICF is from zero to one (0-1), ICF towards one implies one or few plant species are reported to be used by a large fraction of informants to treat a particular type of disease condition. Close to zero implies that informants disagree on which plant to use to treat particular disease conditions (Heinrich et al., 2009; Tounekti et al., 2019). In this case (230-38)/ (230+1) = 0.83 indicates agreement.

3.5.1.4 Language procedures and data processing

A data collection tool written in English and translated to Ateso was used. Bilingual experts translated the tool from English to Ateso for use among the respondents who would don't comfortably use the English tool. A blind back translating team changed the translation to English before pretesting of the translated tool on twenty chicken owners in Kumi district to avoid lack of clarity and areas of ambiguity. Data was collected by a team of Ateso and English speakers by administering questions face to face to the chicken owners. The qualitative data were transcribed and translated by two different people proficient in native ateso and English languages. Themes and codes were developed in QDA miner 6 & Word Stat (PROVALIS). Data was retrieved into elaborate tables. Associated socio-economic demographic quantitative data was entered into MS Excel-spread sheets and imported to Stata 14.2 (https://www.stata.com) and analysed using descriptive statistics.

3.5.1.5 Methodology for KAPs studies

A cross-sectional comparative and descriptive design was used to study the KAPs of the use of EVM in the communities of Soroti district.

3.5.1.6 Sampling and Sample size for KAPs studies

A convenience sample of chicken owners were recruited from six sub-counties of Soroti district. A web calculator (https://www.calculator.net/sample-size-calculator.html) was used to calculate with finite correction. Samples were taken from communities of Soroti district. Data of chicken owners were obtained from the District Production Office (DPO) to make the sampling frames. The following criteria were used; precision rate of 5%, population size of 217,800 and confidence interval of 95%. The calculated minimal sample size required for the study was 384 although 407 (four hundred seven)

was used in effort to increase the sample size for better accuracy. Arapai sub-county (122), Gweri (82), Katine (91), Kamuda (54) and Soroti city (58) respondents.

3.5.1.7 Measures for KAP studies

The questionnaire was research adapted capturing the KAPs of ethnoveterinary use. Information regarding gender, age, residence, highest level of education, sources of ethnoveterinary materials, EVP use and its outcomes were collected. KAPs were measured using the Likert psychometric scale (Joshi et al., 2015). The questionnaire had 16 questions related to knowledge about use of EVM. The knowledge questions were broken down into knowledge of plants, propagation, preparation, concentration, adverse effects and response to adverse effects. The questions were answered on a true/false basis and an "I don't know" option. A correct answer scored one point; an incorrect/unknown answer scored zero points. The total knowledge score ranged from 0 to 16, with a higher score implying a better knowledge of EVM. Attitude towards EVM were measured by 10 questions that were answered on "Agree", "don't agree" and an "I don't know" option. A positive answer scored one point and a negative answer scored zero points. The total attitudes score ranged from 0 to 10, high score implying more positive attitudes. The respondents' practices were measured by 16 questions were answered on "always", "sometimes" and "never" basis (P_1-P_{16}), with 2, 1, 0 scores respectively. The total score ranged from 0 to 32, with a higher score implying better EVM practices.

Data was entered into Micro-soft excel, cleaned and transferred into the Statistical Package for Social Sciences version 26 was used for analysis. Descriptive statistics of frequencies, means, range and standard deviation were determined to establish respondents' demographics. Mean scores, and standard deviation around the mean were calculated for KAPs. Pearson correlation and independent sample t tests were used to detect the relationship between study variables, and linear and hierarchical multiple regression analyses were used to explore correlates of chicken owners' KAPs.

3.5.2 Materials and methods for phytochemical screening of extracts

Extracts from the two most cited plants for anthelmintic action were obtained using the conventional soxhlet extraction method (Hirondart et al., 2020) with minor modifications. Analytical grade ethanol and acetone were the used solvents for each of

the two plant candidates. Ethanol whose polarity is 0.654 was selected to simulate polar water used by chicken farmers, water was not used because the laboratory lacked equipments for removing water from the extracts. Ethanol was also preferred because of its ability to dissolve extracts, low flammability, low toxicity, not affecting the environment and its good preservative properties (Kelber et al., 2017; Sampaio Neto, Batista, & Meirelles, 2018). Acetone was selected because it's only moderately polar with a non-polar methyl group, acetone was used to extract phytochemicals with anthelmintic properties (Bizimenyera et al., 2008). Acetone was considered safe to living cells and a preferred choice in studies involving toxicological assessments (Ishaq et al., 2022; Velázquez-Jiménez et al., 2024).

3.5.2.1 Preparation of crude extracts

Leaves of *Carica papaya* L. (CPL) voucher number SSP/MAK/36 and ripe fruits of *Capsicum annuum* L. (CAF) SSP/MAK/41 were obtained from Soroti district of Uganda during a rainy season (May), auntheticated by Dr. SSegawa Joseph of College of Natural Sciences, Makerere University. Extraction was done using the conventional soxhlet extraction method (Hirondart et al., 2020) with minor modifications. Freshly collected plant material were washed with tap water to remove observable debris. Plant materials were air dried under a shade for two weeks before grinding them to fine powder using a coffee grinder. Ten grams of ground plant material with 5g of pumice stone were placed in a cellulose thimble plugged with cotton. The setup was placed in the conventional soxhlet extraction apparatus containing 300ml of solvent. Extraction was performed using a solid to liquid ratio of 1 to 12 (g/ml) for 8 hours. The extraction was done in duplicate using analytical grade ethanol and acetone as solvents. The extract was concentrated under vacuum using a rotatory evaporator and conserved at 4^{o}C.

3.5.2.2 Qualitative Phytochemical analysis of *Carica papaya* L. and *Capsicum annuum* L.

Saponins: Five millilitres of distilled water was added to 1ml of extract, the solution was filtered. 3ml was added to the filtrate and shahen for 10 minutes, formation of foam that couldn't disappear with addition of HCL was considered positive for saponins (Harborne, 1998).

Tannins: Three drops of lead acetate were added to 1ml of extract, a big white-brown precipitate was indicative of tannins (Archana & Geetha Bose, 2022; Ejikeme et al., 2014).

Alkaloids: Three drops of Meyers reagent were added to 1ml of extract, forming a creamy white precipitate was considered positive for alkaloids (Archana & Geetha Bose, 2022; Chauke et al., 2022)

Flavonosides: Three millilitres of ammonia were added to 2ml extract, then 1ml of concentrated sulphuric acid was added. Yellow coloration in the extract was indicative of flavonosides (Harborne, 1998).

Reducing compounds: About 0.2g of powdered plant sample in 1ml ethanol was added to 3ml distilled water and thoroughly mixed. One millilitre of Fehling's solution A &B in a test tube was boiled and pored into an approximately equal volume of the extract solution. The changes in colour at the bottom of the test tube to red-brown was indicative of reducing compounds(Ramírez-Estrada et al., 2024).

Anthocyanosides: Five millilitres of 1% HCL was added to 1ml of extract, appearance of pink colour showed presence of anthrocyanosides (Sorescu et al., 2018).

Anthraconosides: One millilitre of extract was added to three drops of ammonia solution, a pink precipitate was indicative of anthraconosides (Sorescu et al., 2018).

Coumarins: Three millilitres of 10% NaOH was added to 2ml of extract, formation of a yellow colour was indicative of presence of coumarin (Zohra et al., 2012).

Steroid glycosides: Two millilitres of chloroform was added to equal volume extract and concentrated sulphuric acid, formation of red colour showed presence of steroid glycosides(Kamaraj et al., 2020).

3.5.2.3 Quantitative Phytochemical analysis of *Carica papaya* L. leaves and *Capsicum annuum* L. fruits

i) **Chemicals:** Analytical grade ethanol and acetone were purchased from Vision Scientific & Engineering Uganda Ltd. MS grade acetonitrile and water were supplied by Asinak Lab Equipments & Chemicals, Uganda Ltd. MS grade

formic acid and ricinoleic acid (>99%) were obtained from mox (2% solution of methoxyamine hydrochloride in pyridine), tert-butyldimethylsilyl (TBDMS), N-methyl-N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) and 1% tert-butyldimethylchlorosilane (TBDMCS) were purchased from Vision Scientific & Engineering Uganda Ltd.

ii) **Sample preparation:** Ethanol and acetone extracts of CPL and CAF were prepared by soxhlet method. The sample for GC-MS analysis was prepared as follows; the dried extract was dissolved in 50µL mox and held at 37^{o}C for 1.5h. The derivatization was initiated by adding 50µL N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) and 1% tert-butyldimethylsilyl (TBDMS) followed by incubation at 55^{o}C for 1h. After centrifugation at 13000 rpm for 10min, the supernatant was collected and analysed by GC-MS.

iii) **GC-MS:** (GC-MS- Agilent), model 7000D triple quadruplets equipped with a split injector (Splitless mode) were used. The injection temperature setting was at 250°C and the injected volume was 1µL. The column (ZB-5SMi, 30 m × 0.25 mm × 0.25 µm) was used. The column temperature program was employed in which the initial temperature was 80°C, held for 20 min, followed by a temperature increase at 5°C min/min to 180°C, then held for another 5 min to 250^{o}C, and 15 minutes to 310^{o}C. Helium was employed as the carrier gas at an average linear velocity of 44 .5cm/sec, prime pressure of 500-900. The flow control mode had pressure at 99.8kPa, total flow (50mL/min), column flow (1.46mL/min), linear velocity (44.5 cm/sec), and purge flow (5.0 mL/min). Data were processed on GC-MS and compounds were identified by comparison with the National Institute of Standards and Technology (NIST) in the GC-MS library at 60% match factor cut off levels.

3.5.3 Material and Methods for efficacy studies of the extracts

In vitro efficacy assay of crude extracts (coproculture laboratory experiments) and *In vivo* efficacy experiment (controlled faecal egg reduction tests) in infected chicken on different extract treatments were performed.

3.5.3.1 Management of experimental chickens

Traditionally hatched chicks of indigenous breed from Soroti were left to scavenge and free range with their mother hens so as to expose them to worms in the environment. Faecal samples were collected from the chickens and examined for nematodes infection in the 7th week, the extent of infection was graded and chicken with worm burden of at least 200 eggs per gram of faecal sample were selected for the experiment. The chickens were then set into cages in groups of 3 growers per cage in an area of 0.16 m^2. They were fed on grower mash from Nuvita feeds Uganda Limited and given *adlibtum* tap water. Birds were left to acclimatize in the cages for one week before treatments were done. During the scavenging time no supplementation was provided and no treatments were administered except the Newcastle disease vaccination.

3.5.3.2 Harvesting of *Ascaridia galli* adult helminths

Naturally infected local breed chicks of 7-8 weeks were procured from communities that confessed not using any anthelmentics. The birds were sacrificed and the A. galli collected from the intestines. The worms were washed in PBS, counted and used for in-vitro efficacy assays immediately.

3.5.3.3 *In-vitro* efficacy experiment (Adult helminths motility inhibition assay)

Naturally infected local breed chicks of 7-8 weeks described in section 2.3 above were used as the source of helminths. The birds were sacrificed and the *Ascaridia galli* collected from the intestines in the Faculty of Agriculture and Animal Sciences, Arapai-Soroti (Busitema University). The *Ascaridia galli* from intestines of chicken were washed in PBS (0.01M PBS, 0.138M NaCl, 0.0027M KCl), counted and used for *in-vitro* efficacy assays immediately. The acetone and ethanol extracts were diluted to strengths of 0.32, 0.16, 0.08, 0.04, 0.02 g/ml using (2% DMSO in PBS), ten millilitres (10ml) of the extracts were poured in the petri dishes and 10 worms were added to each petri dish at room temperature (28^{o}C). The number of 10 worms per treatment was determined from www.hedwig.mgh.harvard.edu/sample_size with a power of 0.9, significance level of 0.025 and a minimum detectable difference of 1.2. The probability was 91% that the study would detect a relationship between the independent and the dependent variables at a two-sided 0.05 significance level. About 0.025g/ml of piperazine citrate (interchemie-Holland) was used as a positive control while 2%

DMSO in PBS was used a negative control. The worms were observed for skin damages, motility inhibition and deaths in intervals of 30minutes for 300 minutes. Though worms in PBS were still rigoring at the 300th minute they were weak and the experiments were discontinued at that time for all treatments.

3.5.3.4 *In vivo* efficacy experiment (FECRT)

Twenty-one male chickens for the experiment were selected from those described in section 6.2.3 above. On the day of recruitment, each chicken was kept in isolation for between 30-60minutes or until it voided a faecal dropping. The faecal dropping was then subjected to helminth egg identification and counting as described by Glennon, 2020. Chicken that had stool with helminth egg counts above 200epg were selected for experimental treatments. The selected chicken each weighing about 300-350g were put in cages and kept inside a well-ventilated poultry house. At least three (03) experimental chicken were kept in each cage of 0.16m^2 (Holdsworth et al., 2004) . Commercial growers mash (Nuvita®) was fed with each bird receiving about 55g of the mash daily. Water was given *ad libitum*. The birds were allowed to acclimatise in this condition for seven (07) days prior to experimental treatments. Each chicken was administered with a syringe administered with 1.37g/kg per os of the extract for the extracts groups as a pilot study dose, levamisole was given at 25mg/kg and Piperazine at 100mg/kg. The concentration of the plant extracts were determined from previous *in vitro* experiments as double the lowest concentration that inhibited motility of the highest number of *A. galli* worms. The lowest *in-vitro* concentration that inhibited motility of more than half of the mature *A. galli* was 0.08g/ml of the extract for most extracts, it was doubled to 0.16g/ml *in-vivo* concentration and the birds received 3ml (0.48g) of the extract which was approximately 1.37g/kg. The treatment was repeated on the second day. A week after treatments, pooled faecal samples were collected in air tight plastic vials, the vials were labelled in placed in an ice box for transportation. Faecal samples per treatment were collected for egg count per gram of faeces determination from the Central diagnostic laboratory, College of Veterinary Medicine, Makerere University. Faecal egg count reduction (FECR) and necropsy was done according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) protocols (Saemi et al., 2021; Yazwinski et al., 2003). Egg counts were

determined by modified Mcmaster technique (Glennon, 2020) (Daş, Kaufmann, Abel, & Gauly, 2010).

3.5.3.5 Statistical analysis

The *in-vitro* experiments were conducted in triplicates for each treatment group for the varying concentrations of the extracts, the mean number and standard deviation of paralysed *Ascaridia galli* were determined by one way ANOVA in SPSS 27. The *in vivo* experiments were also conducted in triplicates for each treatment group. The mean and standard deviation of FECR for the different treatment groups were determined by one-way ANOVA followed by Tukey HSD in SPSS 27 to determine the treatment group which was significantly different from others.

3.5.4 Methods and materials for the safety studies in chicken

A pilot acute toxicity study was conducted in chicken. OECD 423 protocol was used with minor modifications. The modificationsa were; not using the four fixed starting dose levels rather the doses of the extracts were standardized at an assumed therapeutic level for a pilot toxicity study. Observation for adverse events was done after the first administration, second administration and daily for the next seven days. Haematological parameters, liver function tests and renal function tests were performed in chicken. Organ histopathology of the heart, kidney and liver were also performed as toxicity indicators.

3.5.4.1 Experimental design

The design was adopted with modifications from the National Research Council (NRC of USA and Canada, 2006). The following IACUC procedures in the annex were followed; the standard operating procedures (SOP) for management of experimental chicken (CH-GZ-001-2022), the SOP for making indoor chicken pens (CH-GZ-002-2022), SOP for the administration of plant extracts to chicken (CH-GZ-004-2022). An experimental design to study the safety of *Carica papaya* L. leaves extract, *Capsicum annuum* L. fruits extract, piperazine citrate and levamisole hydrochloride chicken anthelmintics was conducted by comparing selected organ histopathology, hepato-renal functional tests and haematological parameters. A cohort of indigenous chicks (*Gallus gallus domesticus*) in the Soroti district (Uganda) were left to scavenge with flocks for

six weeks before being selected for caging in the seventh week. In the 7th week, which was also the acclimatisation week, the chickens were fed on standard broiler finisher from Nuvita feeds (Uganda millers limited®) and ad libitum tap water was provided. In the 8th week the chicken were subjected to treatments on 6th and 7th May 2024. The chickens from the different groups were then transported to College of Veterinary Medicine, Animal resources and Biosecurity (COVAB) post mortem laboratory where whole blood samples were collected. Chickens were then euthanized and organs (liver, heart and kidneys) were harvested.

3.5.4.2 Management of study chickens

Traditionally hatched chicks of indigenous breed were left to scavenge free-range with the hens so as to expose them to nematodal parasites in the environment. Faecal samples were collected from the chickens and examined for nematode infection in the 7th week. The extent of infection was graded and chickens with a worm burden of at least 200 eggs per gram of faecal sample, and weighing between 300 and 350 grams were selected for the experiments. The chickens were then put into cages in groups of three chickens per cage, with each cage having an area of 0.16 m^2 in FAAS, Busitema University farm. They were fed on grower mash from Nuvita feeds (Uganda millers Limited®) and given *adlibtum* tap water. The detail of the composition of the feeds is shown in **Table 1**. Birds were left to acclimatize in the cages for one week before treatments were given. During the free-ranging period, no supplementation was provided, however the birds received vaccination against Newcastle disease.

Table 1: Composition of grower finisher feed used in the study

Ingredient	Composition	Ingredient	Composition
Crude Protein (**%**)	18	**Vitamins (per kg)**	
Metabolizable Energy (**Kcal/kg**)	3150	Vitamin A (**I.U**)	8000
Calcium (**%**)	0.89	Vitamin D_3 (**I.U**)	3500
Available Phosphorus (**%**)	0.38	Vitamin E (**I.U**)	50
Sodium (**%**)	0.2	Vitamin K (**I.U**)	3
Methionine (**%**)	0.38	Thiamine (**mg**)	4
Methionine + Cystine (**%**)	0.75	Riboflavin (**mg**)	5
Lysine (**%**)	1.0	Pyridoxine (**mg**)	4
Threonine (**%**)	0.55	Pantothenic acid (**mg**)	14
Tryptophan (**%**)	0.18	Folic acid (**mg**)	1
Arginine (**%**)	1.1	Biotin (**µg**)	100
Valine (**%**)	0.56	Niacin (**mg**)	40
Leucine (**%**)	0.9	Choline (**mg**)	400
Isoleucine (**%**)	0.55	Vitamin B_{12} (**µg**)	12
Histidine (**%**)	0.28		
Phenylalanine (**%**)	0.6		
Trace minerals (**per kg**)			
Manganese (**mg**)	70		
Iron (**mg**)	20		
Copper (**mg**)	8		
Zinc (**mg**)	70		
Iodine (**mg**)	0.5		
Selenium (**mg**)	0.3		

Source: Nuvita feeds, Jinja City in Uganda.

3.5.4.3 Administration of treatments

Extracts were prepared as described in section **3.5.2.1.**

Seven separate treatment groups were setup in triplicate; CAFa, CAFe, CPLa, CPLe, Piperazine citrate, Levamisole hydrochloride and 0.2% DMSO. Making a total number of twenty one chickens. The lowest concentration of compound that paralyzed the

highest number of *Ascaridia galli* worms in the *in-vitro* experiments (0.08 g/ml) was doubled to determine the dose of the extracts that was used in chickens (Zirintunda et al. 2025). This was to simulate the discriminating concentration concept. The extracts were administered in 3 ml volumes (0.48 g) per chicken per day (1.37g/kg body weight). Piperazine citrate was given at 100 mg/kg body weight as recommended by the manufacturer and levamisole hydrochloride at 25mg/kg body weight, also as recommended by the manufacturer. DMSO (0.2%) in phosphate buffered saline (PBS) was administered as negative control. The treatment was repeated on the following day. The birds were transported in plastic baskets in the night a week after treatments and allowed to rest for four hours in the post-mortem room. Birds were sacrificed in the post mortem room of the College of Veterinary Medicine, Makerere University.

3.5.4.4 Observation for adverse events

The chickens were observed after the first administration, second administration and daily for seven days thereafter. They were observed for lethargy, diarrhoea, dropping saliva, ruffled feathers, lack of appetite and deaths.

3.5.4.5 Organ collection and histological slide preparation

The chickens were euthanized by mechanical cervical dislocation (Boyal et al., 2020). Chickens were sacrificed by severing the cervical region using a surgical blade. Post-mortems were conducted in the pathology laboratory of COVAB and the target organs (heart, kidneys and liver) were collected in 10% neutral buffered formalin solution for 48 hrs. The fixed specimens were trimmed and placed in labelled cassettes, then placed in formalin again and finally transferred into the tissue processor. The tissues were processed in a semi-automated processor microtome to achieve very thin tissue slices which were transferred to glass slides, based on an established procedure (Dey, 2018). The slice sections were stained with haematoxylin & eosin standard protocols (Feldman & Wolfe, 2014). The slides were observed at a magnification of times two hundred (X200) in the COVAB central diagnostic laboratory guided by a pathologist (Dr. Mathias Afayoa).

3.5.4.6 Biochemical analysis

Blood urea nitrogen (BUN), sodium (Na^+), chloride (Cl^-) and creatinine (Cre) were determined using a semi-automatic clinical analyser DIRUI-7000 (Jilin Jingquan Medical equipments Limited®), Changchun- China. Uric acid and the liver function parameters; Aspartate aminotransferase (AST), Alanine transaminase (ALT), Glutamyl transferase (GT), Total bilirubin (TB), Direct bilirubin (DB), Total proteins (TP), Albumin (ALBU) & Globulins (Globu) were determined using Cobas® C311 analyzer (Roche, Germany).

3.5.4.7 Haematological assessment

Blood was collected by brachial vein puncture (Kelly & Alworth, 2013). A bird was restrained by an assistant and the wing was stretched out to form a V-shape. Obstructing feathers were removed and the area was sanitized by swabbing area with 70% alcohol. Blood was drawn using a 20-gauge needle and ensuring blood collection not exceeding 1% of the total body weight of the bird. The needle was withdrawn gently and a gentle pressure applied to the area to stop bleeding. The needle was removed and the blood was gently deposited into labelled pink top tubes containing EDTA. Gentle inversion was done to mix the blood with the anticoagulant, the blood was temporarily stored in ice boxes pending tests which were performed on that day.

Manual blood smear review (MBSR) and manual differential leukocyte counts (MDLC) were done as described by Comar et al. (2017). Meticulous examination of well-prepared stained blood smears with keen assessment of any morphological changes was conducted by a team having 10 to 40 years' experience in MBSR, in the central diagnostic laboratory of the College of Veterinary medicine and Biosecurity, Makerere University.

3.5.4.8 Statistical analysis

The liver function, renal function, haematological and biochemical datasets were entered in MS-excel for cleaning and transferred to SPSS version 26 for analysis. Descriptive statistics was done to obtain the mean, median, standard deviation and confidence intervals. Two-way ANOVA was done followed by Tukey's HSD multiple comparisons. Statistical significance was considered at $p \leq 0.05$.

3.5.4.9 Histopathological assessment

The protocols were adopted with modifications from the Organisation of Economic Cooperation Development (OECD, 1996). The Renal function tests, liver function tests and the haematology parameters of the treatment groups were compared to their control group; a treatment having one or more aspects significantly different was considered toxic. The significance levels and the number of significant aspects showed the toxicity levels. For organ histopathology; the heart, kidney, liver of the three birds per treatment were assessed. The number of organs with observable lesions over the total number of organs per treatment was the scoring method (Gibson-Corley et al., 2013). Having no observable lesions for a treatment (0/9) was considered safe, having one organ with observable lesions (1/9) was considered moderate and having more than one organ with observable lesions was considered toxic (safe<1/9>toxic).

3.6 Ethical Considerations

i. Proper care of chicken during the experiments by having a clean housing. Chickens were provided with water and feeds.
ii. Having an animal attendant for immediate reporting of emergencies and having isolation sections for chicken that would require isolation.
iii. Beneficence was by having complete safety gears for the research team outlined as facemasks, overalls, hand gloves and gum boots.
iv. Acquiring institutional ethical review certificates and approvals. All procedures that involved animals were approved by the Institution Review Board (IRB) or Institution Animal Care and Use Committee (IACUC) of the School of Veterinary Medicine and Animal Resources (SVAR), an ethical review certificate was acquired from the School of Veterinary Medicine and Animal Resources (SVAR_IACUC /93/2021). The study followed the guidelines of the Uganda National Council for Science and Technology (UNCST) of March 2007 page 21-29. The guidelines for research during the COVID 19 pandemic concerning safety of respondents (UNCST, 2020) were followed. Approvals were given by the UNCST under the reference number A220ES. Permission to conduct research was also granted by the Soroti District Veterinary Office.
v. The study aim, scope, benefits and rights of the respondents were clearly spelt at the beginning of the questionnaires. It was clearly spelt that participating was

absolutely voluntary and the respondents were free to discontinue the engagement at any time. A data collection tool written in English and translated to Ateso was used. Consent forms included explanation of the study to the chicken owners before signing in consent. Confidentiality was assured by omitting identity information of the respondents.

vi. The veterinary pathologists that did necropsies did not participate or have any idea on the blinding of the study animals.

3.7 Quality Control of the Research Work

Quality control was ensured under the following guidelines

3.7.1 Reproducibility of the Research

The experiments were triplicated with relevant controls to ensure reproducibility by other researchers. The protocols were assessed by various ethical certifying bodies to ascertain reproducibility and quality.

3.7.2 Relevance of the Research

The experiment were to show the efficacy and safety of the ethnoveterinary compounds in use in the Soroti poultry hub. The phytochemicals were identified and quantified. This was important because synthetic anthelmintics are expensive and resistance has already been reported against some synthetic anthelmintics. Synthetic poultry anthelmintics leave drug residues which enter the food chain and cause public health concerns. This research purposes to inform the decision makers of a cheap and possibly safer alternative chicken helminths drugs. The study augments achieving part of sustainable development goals (SDGs) one and two. The study is in line with the National Research and innovation Programme (NRIP) Framework under the ministry of Science, Technology and innovations (NRIP, 2019; UNCTAD/DTL/STICT/2020/4) and the Makerere University graduate research agenda therein the strategic research, innovations and commercialization plan 2021-2030.

3.7.3 Responsiveness of the research

This is the ability to quickly detect systematic mistakes and correct them (Hubbard& Carriquiry, 2018; Munafo et al., 2017). This was ensured by registering the research

under the Uganda National Council of Science and Technology. This was done to ensure transparency of data by ensuring clear access to primary data. Even proper use of *p-values* and the Null Hypothesis significance testing (NHST) with no publication bias was ensured. The statistical methods showed the understanding of the limitations of the *p-values* and NHST in explaining outcomes and probabilities.

3.8 Study Limitations

i. Inaccuracies of translation of the guiding questions from English to Ateso
ii. Not all the mentioned plants were tested for efficacy and safety
iii. Only two solvents were used to extract compounds from plants
iv. Use of one species of helminths in testing efficacy
v. Variations in the state of minds of the respondents
vi. Ethnoveterinary measures lack a clear statistical and pharmacological rational.
vii. Lethal doses of the extracts in chicken were not determined

CHAPTER FOUR

DOCUMENTING THE ETHNOVETERINARY KNOWLEDGE, ATTITUDES AND PRACTICES FOR CHICKEN HELMINTH CONTROL

4.1 Introduction

Ethnoveterinary practices are when indigenous knowledge of nursing, training and healing are used in managing animals (Rahman et al. 2022) and when these practices are readily available (Xiong & Long, 2020). The use of EVM is said to be as old as human existence but is not well documented especially in the areas of Africa and Asia who are the main users (Dzoyem et al., 2019). Over the years of chicken keeping; farmers have identified a pool of plants and practices which are used to manage chicken diseases and this is passed from one generation to the next (Gueye EF, 1999; Marandure, 2016; Masimba et al., 2011; Misra & Kumar, 2004; Moreki et al., 2010; Njau, 2001; Olanipekun & Tedela, 2013; Petrus et al., 2011; Uncini et al., 2001; Yigezu et al., 2014). Over 20,000 species are used as medicine in the world (Jamil et al., 2022). The local ethnoveterinary knowledge has the advantages of being cheap, organic, acceptable and sustainable (Uprety et al. 2022).

Information on ethnoveterinary knowledge is not widely documented despite the increased use of these alternative medicines in the control of chicken diseases (Amoia *et al.,* 2021). Ethnoveterinary knowledge is passed to generations through verbal communications which are rarely converted into durable records (Yineger *et al.*, 2007).

Soroti district was selected because it has a high number of households that keep chicken and it is a commercial centre for local chicken in Uganda. Although related studies have been done in livestock and humans no explicit study has been done on the use of EVM in chicken. Other users have started even packing and marketing the undocumented and obscure materials.

This research purposed to consolidate data on EVM in control of chicken helminths by; documenting the ethnoveterinary knowledge for chicken helminths control and documenting the specific practices for the control of chicken helminths. The study also purposed to document the implications of ethnoveterinary knowledge, attitudes and practices in the control of chicken helminths.

4.2 Materials and Methods

(Described in section **3.4** &**3.5.1** of chapter 3)

4.3 Results

4.3.1 Study of ethnoveterinary Practices for chicken helminths control

4.3.1.1 FGDs transcript section on practices for treatment chicken helminths

The participants of FGDs mentioned various plants that they claimed to be effective against chicken helminths as in quotes below;

1. *Pawpaw tree roots (Carica papaya L.), jetropha roots (Jatropha curcas L.) and red pepper fruits (Capsicum annuum L.) treats chicken worms*

The processes of preparation were almost uniform for different plant materials claimed to be effective against chicken worms. The materials are crushed and water is used as solvent as mentioned in quotes below;

2. *Black grain (Chamaecrista nigricans (Vahl) Greene), tobacco. I pound cocoa seeds (Theobroma cacao) and mix in water for birds to drink. Pawpaw roots pound and added to water. Onions (Allium cepa L.), pound and added to water. I use Aloevera as prophylaxis, it works against helminths although I use it against all infections.*
3. *We mix red pepper in most of the mentioned plants and worms get cleared, we don't have a program targeting the worms but once the chicken take our mixtures we don't get any indicators of worm burdens. We use Gambian indigo (Philenoptera laxiflora (Guill. & Perr.) Roberty), pawpaw and Marijuana.*
4. *Red pepper is dangerous to worms, however we also use wild Aloevera, turmeric, local soda ash, neem tree leaves and Uganda green heart barks. Bermuda grass (Cynodon dactylon (L.) is effective against worms and NCD. We also use Uganda coral roots, we remove the barks, pound and soak in drinking water. Black grain is effective against worms, even tarmarind leaves when pound and added to drinking water. We pound turmeric rhizomes and soak in water. Neem tree leaves are pounded and soaked in water.*

4.3.1.2 Chicken owners' helminth control plan

Only 13/78 (16.7%) agreed that they were deworming chicken every three months, the rest had various arrangements as follows No response 7/78 (9%), rarely or doesn't 16/78 (20.5%), When chicken are sick 9/78 (11.5%), after 1-2 months 25/78 (32.1%) after 3 months 13/78 (16.7%), after 4 months 2/78 (2.6%), after 5 months 1/78 (1.3%), Twice a year 4/78 (5.1%), Rainy season 1/78 (1.3%). Thirty nine out of seventy-eight (39/78) agreed that they were using herbal anthelmintics, 29/83 (37.2%) synthetic, 12/83 (15.4%) both herbal and synthetic and 3/78 (3.8) didn't respond.

The information on ethnoveterinary Practices for treatment of chicken helminths in Soroti district was used to make **Table 2.** Twenty-eight (28) plant families with 39 species were cited. The top-ranking plants were *Capsicum annuum* L. (PRK = 65.4%) followed by *Carica papaya* L. (PRK = 42.3%). The most cited family being Solanaceae followed by Caricaceae. Leaves were mostly used followed by barks. The herbs are prepared by pounding or crushing and soaking in cold water for 1-6 hours, and administered orally to the chickens.

Table 2: Ethnoveterinary Practices for treatment of chicken helminths in Soroti district

Vernacular name (Ateso)	Plant name	Plant species	Family	Voucher Number	Plant part	Preparation	Citation	PRK (%)	FC (%)
Ecucuka	Aloevera	*Aloe barbadensis* Miller	Aloeaceae	SSP/MAK/23	Leaves	Crushing	20	25.6	8.7
Ecucuka	Fez aloe	*Aloe peglerae*	Aloeaceae	SSP/MAK/28	Leaves	Crushing	3	3.8	1.3
Emutungulu simu	Garlic	*Allium sativum* L.	Amaryllidaceae	SSP/MAK/58	Blub	Crushing	3	3.8	1.3
Ebwolo	African custard apple	*Annona senegalensis* Pers.	Annonaceae	SSP/MAK/45	Bark	Pounding	1	1.3	0.4
Emulondo	Tonic root	*Mondia whytei* (Hook.f.) Skeels	Apocynaceae	SSP/MAK/55	Roots	Pounding	1	1.3	0.4
Eligoi	Yellow cleander	*Thevetia peruviana* (Pers.) Schum	Apocynaceae	SSP/MAK/26	Leaves	Crushing	4	5.1	1.7
Equinini	Dwarf Mexican Marigold	*Schkuhria pinnata* (Lam.) O. Ktze.	Asteraceae	SSP/MAK/10	Whole plant	Pounding	1	1.3	0.4
Enanasi	Pineapple	*Ananas comosus* (L.)	Bromeliaceae	SSP/MAK/54	Fruits, leaves	Crushing	1	1.3	0.4
Epopong	Cactus	*Nopalea conchenillifera*	Cactaceae	SSP/MAK/64	Leaves	Crushing	1	1.3	0.4
Epeduru yendidin	Black grain	*Chemaecrista nigricans* (Vahl) Greene	Caesalpinioideae	SSP/MAK/16	Whole plant	Crushing	21	26.9	9.1
Epeduru	Tamarind	*Tamarindus indica* L.	Caesalpinioideae	SSP/MAK/21	Leaves, bark	Pounding	8	10.3	3.5
Abacci	Ugandan green heart	*Warburgia ugandensis* Sprague subsp.ugandensis	Canelaceae	SSP/MAK/29	Bark	Pounding	10	12.8	4.3
Ejaye	Marijuana	*Cannabis sativa* L.	Cannabaceae	SSP/MAK/60	Leaves, seeds, roots	Crushing	8	10.3	3.5

Epapaile	Pawpaw	*Carica papaya* L.	Caricaceae	SSP/MAK/36	whole plant	Pounding	33	42.3	14.3
Ekulonyo	Terminalia	*Terminalia schimperiana (Engl.) Diels*	Combretaceae	SSP/MAK/72	Leaves	Pounding	1	1.3	0.4
Ekoropot	Wondering Jew	*Commelina benghalensis* L.	Commelinaceae	SSP/MAK/52	Leaves, stems	Pounding	1	1.3	0.4
Esuju	Pumpkin	*Cucurbita moschata* L.	Cucurbitaceae	SSP/MAK/74	Seeds, leaves	Pounding	1	1.3	0.4
Ejumula	Jatropha	*Jatropha curcas* L.	Euphorbiaceae	SSP/MAK/34	Leaves	Crushing	1	1.3	0.4
Ekoko	Cocoa	*Theobroma cacao*	Malvaceae	SSP/MAK/67	Seeds	Crushing	1	1.3	0.4
Elira	Chinaberry	*Melia azedarach* L.	Meliaceae	SSP/MAK/43	Leaves, bark	Pounding	4	5.1	1.7
Neem	Neem tree	*Azadirachta indica* Juss.	Meliaceae	SSP/MAK/31	Leaves	Crushing	14	17.9	6.1
Etekwa	Light wood	*Albizia coriaria* Oliv.	Mimosoideae	SSP/MAK/14	Leaves, bark	Pounding	2	2.6	0.87
Ekisimu (a)	Apple ring acacia	*Acacia albida* Delile	Mimosoideae	SSP/MAK/04	Leaves, bark	Pounding	1	1.3	0.4
Ekisimu (b)	Shittim wood	*Acacia hockii* De wild.	Mimosoideae	SSP/MAK/44	Leaves, bark	Pounding	1	1.3	0.4
Emoringa	Moringa	*Moringa oleifera* Lam.	Moringaceae	SSP/MAK/07	Leaves	Crushing	2	2.6	0.87
Ekalitusi	Saligna gum	*Eucalyptus grandis* Maiden	Myrtaceae	SSP/MAK/33	Leaves	Crushing	2	2.6	0.87
Engosorot	Uganda coral	*Erythrinica abyssinica* DC.	Papilionaceae	SSP/MAK/24	Bark	Pounding	4	5.1	1.7
Ekaka	Gambian indigo	*Philenoptera laxiflora* (Guill. &Perr.) Roberty	Papilionaceae	SSP/MAK/02	Bark	Pounding	1	1.3	0.4
Ekosile	Giant rat tail's grass	*Sporobolus pyramidalis* Beauv.	Poaceae	SSP/MAK/08	Roots	Pounding	1	1.3	0.4
Emuria	Bermuda grass	*Cynodon dactylon* (L.) Pers.	Poaceae	SSP/MAK/51	Leaves, stems	Pounding	2	2.6	0.87
Eleketete	Portulaca	*Portulaca quadrifida* L.	Portulacaceae	SSP/MAK/30	Whole plant	Crushing	9	11.5	3.9

Usuk	Sand knobwood	*Zanthoxylum leprieurii* Guill. & Perr.	Rutaceae	SSP/MAK/06	Bark	Pounding	3	3.8	1.3
Ekele	Abyssinian Rose	*Harrisonia abyssinica* Oliv.	Samourabaceae	SSP/MAK/27	Leaves	Crushing	1	1.3	0.4
Emulalu	Redpepper	*Capsicum annuum* L.	Solanaceae	SSP/MAK/41	Fruits	Crushing	51	65.4	22.2
		Capsicum frutescens L.	Solanaceae	SSP/MAK/53	Fruits	Crushing	3	3.8	1.3
Etulelut	Soddom apple	*Solanum incanum* L.	Solanaceae	SSP/MAK/40	Leaves, fruits.	Make ash from dried materials	1	1.3	0.4
Etaaba	Tobacco	*Nicotianum tabacum* L.	Sonanaceae	SSP/MAK/17	Leaves	Crushing	4	5.1	1.7
Ebisali	Tumeric	*Curcuma longa* L.	Zingiberaceae	SSP/MAK/48	Rhizome	Crushing	3	3.8	1.3
Etangawuzi	Ginger	*Zingiber officinale* Rosco.	Zingiberaceae	SSP/MAK/59	Rhizome	Crushing	1	1.3	0.4

4.3.2 Implications of knowledge, attitudes and practises of Ethnoveterinary control

4.3.2.1 Sociodemographic characteristics of respondents

Results indicated that majority of the respondents were male. Majority of respondents were aged 21-40 years and almost equal numbers having attained primary and secondary education as their highest level (**Table 3**).

Table 3: Socio-demographic characteristics of respondents to KAPs questionnaire

Characteristic	Sub-group	Frequency	%
Sex	Males	304	75
	Females	103	25
Age in years	Below 20	23	5.6
	21-40	269	66
	41 and above	115	28
Sub-counties	Arapai	114	28
	Gweri	73	18
	Katine	92	23
	Kamuda	66	16
	Soroti city	62	15
Highest level of Education	Primary	138	34
	Secondary	136	33.4
	Postsecondary	133	32.6

Most respondents were males, 21-40 years and had attained secondary and post secondary education.

4.3.2.2 KAPs towards EVM by chicken owners

The Soroti chicken owners showed a substantial EVM knowledge capacity. The mean knowledge score was 11.6 out of 16 (SD =3.5), ranging from 0 to 16. They also held positive attitudes towards the use of EVM in chicken treatment. The mean attitudes score was 7.8 (SD = 1.95), ranging from 0 to 10. The mean score on practices scale was 16.39 (SD = 5.58), ranging from 0 to 32, **Table 4 and Appendix (iii)**. In order to make KAPs scale comparable, standardized mean scores were calculated. The results were 72.5, 78 and 51.2 for the KAPs scales respectively. Using an independent sample *t* test,

there was no significant gender difference in any of the KAPs scores (p>0.05). Using Pearson's correlation analysis, there was significant relationship between age and Knowledge (p <0.05), age and attitudes (p < 0.001), Age and practices (p <0.001). There was a significant relationship between education and knowledge (p <0.001), there was no significant relationship between education and attitudes even education and practices (p > 0.05). There was a significant relationship of residence with knowledge scores (p < 0.001), attitudes scores (p < 0.001) and practices scores (p < 0.001).

Table 4: Descriptive statistics and reliability of the study scales

Scale	No. Items	Mean	Median	SD	Range	Cronbach's α
Knowledge	16	11.6	12	3.5	0-16	0.806
Attitudes	10	7.8	8	1.95	0-10	0.681
Practices	16	16.39	16	5.58	0-32	0.830

The mean knowledge score was 11.6 with 80.6 % reliability of the items, the mean attitudes score was 7.8 with 60.1% reliability of the items, the mean practice score was 16.39 with 83% reliability of the items.

4.3.2.3 Predictors of Chicken owners' knowledge of EVM

Multiple regression was done to assess predictors of chicken owners' knowledge of EVM. Knowledge total score was entered as an outcome variable, chicken owners' gender, residence, age and education were entered as potential predictors. The results showed that generally the model was not able to predict significant proportion of chicken owners' knowledge of EVM in chicken F (4, 407) = 1.808, p = 0.126, ΔR^2= 0.008. Residence alone was a significant predictor of chicken owners' knowledge (p = 0.035). **Appendix (iv)** shows the model fit.

4.3.2.4 Predictors of Chicken owners' attitudes on EVM

Hierarchical multiple regression analysis was run to assess chicken owners' knowledge of EVM could predict their attitudes beyond their gender, residence, age and education. The first model (having chicken owners' gender, residence age and education) could predict the chicken owners' attitudes, F (4, 407) = 2.686, p = 0.031, ΔR^2= 0.016.

Residence alone could significantly predict chicken owners' attitudes on EVM, P = 0.003. The second model showed that chicken owners' knowledge of EVM significantly predicts their attitude toward EVM, F (5, 407) = 22.83, $p = 0.001$, $\Delta R^2 = 0.212$. Almost 21% of the variability in chicken owners' attitudes could be explained by their knowledge level. **Appendix (v)** shows the model fit.

4.3.2.5 Predictors of Chicken owners' practices of EVM

Hierarchical multiple regression analysis was done to assess if chicken owners' attitudes towards EVM could predict their practices above and beyond the gender, residence, age, education and knowledge. The first model (having chicken owners' gender, residence, age, education and knowledge could significantly predict chicken owners' practices F (5, 407) = 10.06, $p = 0.000$, $\Delta R^2 = 0.100$. Knowledge alone could significantly predict chicken owners' EVM practices $p = 0.000$. Age alone could also predict chicken owners' EVM practices $p = 0.013$, with older people practicing EVM than young ones. The second model showed that chicken owners' attitudes towards EVM significantly predict their practices F (6, 407) = 11.035, $p = 0.000$, $\Delta R^2 = 0.129$. Almost 13% of the variability in chicken owners 'practices could be explained by their attitudes towards EVM. **Appendix (vi)** shows the model fit.

4.4 Discussions

4.4.1 Ethnoveterinary Practices for chicken helminths control

Most of the farmers were able to state the signs that can be used to identify chicken with helminths. The farmers can make informed decisions especially when helminthiasis manifests clinically. Some farmers (20%), do not deworm their chicken; such an inclination affects production and productivity manifesting as low weight gains and high FCR (Shifaw et al., 2021; Van et al., 2020a). The deworming regimes vary from one farmer to another usually not having the lifecycle of the helminths in consideration. About 5.1% (4/78) deworm chicken twice a year, 1.3% 1/78 deworm chicken after 5 months, 2.6% 2/78 deworm after 4 months. Although the regime depends to the duration of action, pharmacokinetics and pharmacodynamics of the dewormer, such time intervals are too long for a helminths of two to three months lifecycle (Macklin & Hauck, 2019). About (16.7%) 13/78 deworm after every three months, this would be the recommended based on the rational of the helminth's

lifecycles. About (32.1%) 25/78 deworm after every one to two months, this is an overuse of anthelmintics which could lead to accumulation of drug residues in chicken. Overuse of low doses of drugs with anthelmintic activity could also lead to development of resistance to anthelmintics (Fissiha & Kinde, 2021b). Anthelmintic resistance has already been reported in chicken in Uganda and it's not known whether herbal drugs have contributed to this problem.

Many chicken farmers use herbal approaches of unknown methods of action and longevity of effect. There is need to establish if the repeated use of herbal anthelmintics is associated with the developing of resistance to synthetic anthelmintics. About (47%) 39/83 of the farmers use herbal anthelmintics, (34.9%) 29/83 use synthetic anthelmintics and 14.5% (12/83) use both herbal and synthetic anthelmintics. This shows that herbal anthelmintics are acceptable and considered effective, this is in agreement with (Jato et al., 2022; Liu et al., 2020). Herbal anthelmintics are usually cheap and accessible compared to the synthetic formulations which may be prohibitively expensive or not accessible (Jato et al., 2022). The practice of alternating use from synthetic to herbal anthelmintics or even vice versa is thought to be a result of the futile trials with any. The approach of using herbal and synthetic concurrently is not known and requires further study because no plant-synthetic synergistic effects have been mentioned to this date.

Capsicum annuum L. (Solanaceae) has phytochemicals with anthelmintic properties (Farooq et al., 2008). Family Solanaceae has very many species which are effective against helminths because their fruits contain capsaicinoids (Gentiles et al., 2019). The family has been reported to have plant species that are effective even against chicken tapeworms (Nagi et al., 2024; Obeng et al., 2022). Various parts of *Carica papaya* L. (Caricaceae) are reported to contain compounds that can destroy helminths (Dotto & Abihudi, 2021). The different parts of the Caricaceae plants have been reported to be effective against helminths (Goku et al., 2020). Extracts of *Chemaecrista nigricans* (Vahl) Green were reportedly effective against helminths (Osunga et al., 2023). Aloe extracts are said to have lethal action against helminths (Shelke et al., 2020). The extracts of *Azadirachta indica* Juss. (Meliceae) had promising anthelmintic properties (Joshi, 2022). *Warburgia ugandensis* Sprague subsp.ugandensis (Canelaceae) extracts are effective against helminths only that their safety is still unknown (Liu et al., 2020).

Portulaca quadrifida L. (Portulacaceae) have many pharmacological benefits including having anthelmintic action (Ahmadou et al., 2022).

The used plant parts mentioned as anthelmintics were Bark, stems, seeds, fruits, flowers and roots. However, leaves were the most mentioned parts. The phytochemical compositions vary with season of the year, nature of soils and stage of growth of the plants however phytochemicals tend to be concentrated in barks and leaves compared to other parts (Patle et al., 2020).

The preparation methods were crushing or pounding and adding water or soaking in water, this is simulated to using of a polar solvent in extraction. However, *Solanum incunum* was dried, burned to make ashes that were added to drinking water as anthelmintic, this method of preparation and use is different from what is common in literature. Anthelmintic properties of the extracts of Solanaceae are reported not their ashes (Ono et al., 2021).

4.4.2 Implications of knowledge, attitudes and practises of Ethnoveterinary control

Though use of EVM could be as old as the human race, the KAPs of use change with time. Though related studies have been carried out in other areas, this is the first study in Soroti district focussing on chicken disease control.

4.4.2.1 Chicken owners' knowledge

Chicken owners showed commendable knowledge of use of EVM in managing chicken diseases (average mean knowledge score of 72.5%). This is in agreement with (Ssenku et al. 2022; Schultz et al. 2020; Ebifa-Othieno et al. 2017; Ojelel et al. 2019) who found that people had good ethno-medicine knowledge bases. Most farmers 308 (77%) knew that though plant alternatives have advantages but could lead to adverse effects during use. Majority of the chicken owners were able to propose the causes of adverse effects. Majority expressed knowledge of preparation, propagation and administration. The model couldn't predict chicken owners' knowledge although residence alone could significantly predict the chicken owners' knowledge ($P<0.05$), Table 4. There was no significant gender difference in knowledge because all gender has access to ancestors and peers as sources of EVM information. Although there was no significant residence

difference in knowledge, chicken owners from rural sub-counties tended to be more knowledgeable compared to those from urban settings possibly because of their easier access to various medicinal plants. The urban chicken owners are more likely to access conventional chicken drugs compared to their rural counterparts. The good scope of knowledge implies the effectiveness of family informal trainings and how EVM is esteemed by communities. This also indicates the interest and the acceptability of EVM. Participatory approaches of identifying alternative chicken medicines are recommended since chicken owners have empirical experiences of the plant alternative medicines.

4.4.2.2 Chicken owners' Attitudes towards EVM

Chicken owners were convinced about the action of EVM against chicken diseases. Majority of the chicken owners feel that EVM are useful alternatives to conventional chicken medicines (average mean attitude score of 78%). This is in agreement with (Ebifa-Othieno et al. 2017; Gumisiriza et al. 2021; Abera& Mulate, 2019) who found the people agreeable with the use of ethno-medicine. The chicken owners were satisfied that EVM are safer than conventional chicken medicines to chicken and the final consumers. EVM are appropriate alternatives for people that opts for organic chicken farming. Model 1 would significantly predict chicken owners 'attitudes ($P< 0.05$), Table 5. Residence alone could predict chicken owners' attitudes ($P<0.01$). Knowledge was a significant predictor of attitudes ($P<0.01$), as seen in Model 2. There was no gender difference in attitudes because all accept and use EVM. Chicken owners from rural sub-counties showed better attitudes towards EVM compared to those from urban settings. Education doesn't affect the use of EVM in chicken because it's generally considered effective and acceptable. Chicken owners are willing to promote EVM regardless of the inculcated myths and fallacies. This alternative medicine is hinged on particular traditions which are usually more trusted than synthetic drugs. The observed attitudes as expressed by willingness to preserve, plant, promote and support research in EVM is an indicator that the practice is esteemed. Teaching the principles of ethnoveterinary medicine across various platforms can cause more people to have positive attitudes on this alternative. This implies that if processed production could be scaled up then more people would adopt these alternative medicines.

4.4.2.3 Chicken owners' Practices

About half the population practice EVM (average practice score of 51.2%), this is in agreement with (Chaturvedani et al. 2016; Adeleye et al. 2021; Sharma et al. 2022) who found the use of ethno-medicine as a common practice. Although chicken owners know and are positive about the use of EVM yet not many are willing to practice it. The products are not processed, they contain other undesired substances which could have toxic effects. The common adverse effects make the users very careful or even fail to use EVM but would have used if the products were processed and rendered empirically safe.

Model 1 could predict the practices of chicken owners' ($P<0.01$). Age alone could predict the practices of chicken owners' ($P<0.05$), older people use EVM compared to young ones. Knowledge alone could predict the practices ($P<0.01$), knowledge determines effective preparations and use. Model 2 could predict the practices ($P<0.01$). Attitudes could predict the practices ($P<0.01$), those who are convinced by the effectiveness and advantages are the one most likely to use EVM.

4.4.2.4 Prospects of EVM as future Chicken Medicines

Alternative plant medicine will remain useful because of antimicrobial resistance and risks of synthetic drug toxic residues in the products of chicken. The knowledge of the chicken farmers will be harnessed as baseline for development of new drugs, used on organic farms and green synthesis of drugs. The future of EVM will depend on the speed of identifying and isolation of phyto-active compounds from plant candidates. Clinical trials for efficacy and safety of the specific active metabolomes will be the next prudent step in conjunction with studying of the most appropriate packaging. There is also need to assess the possible synergistic actions of EVM with synthetic drugs. Legislation of regulation and use will have to be reviewed as the products approach national and international markets. Increased use of EVM will require medicinal plants planting and gazetting major sources.

It's likely that some plants have anthelmintic properties apparently based on the consensus theory. This is presumptuous especially for the plants with the highest ICF (pawpaw and red pepper). However, there is need for experiments on all the mentioned plant candidates because empirical research is more rational than untested claims of

consensus. Phytochemical screening would be necessary for the different plant parts in the varying seasons of rain and sunshine.

Experiments of the effects of the plant candidates on helminths can provide an opportunity against the emerging resistance against synthetic anthelmintics. Single plant alternatives or plant combinations can be used to generate data to advise the national drug authority (NDA), Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) and drug designing companies. However proper testing for the efficacy and safety of the candidates is required regardless of the ICF of the plant candidate.

4.4.2.5 Conclusion

Chicken owners demonstrated a good knowledge base and were able to use EVM as alternative chicken medicine. The chicken owners were convinced about the effectiveness of EVM. EVM were acceptable by the various chicken owners' gender, age groups and educational levels. Even with the good average knowledge and attitude score, the average practice score was low. The findings are in line with the age, gender and dynamics of knowledge hypothesis (Albuquerque et al. 2011) and the urbanisation & knowledge loss hypothesis (Sogbohossou et al. 2015). Although the results are informative but a detailed KAPs study outlining the EVM for specific chicken diseases is recommended.

CHAPTER FIVE

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL COMPOSITION OF *CARICA PAPAYA* L. AND *CAPSICUM ANNUUM* L. MEDICINAL PLANTS

5.1 Introduction

The use of ethnoveterinary medicines is said to be as old as human existence but is not well documented especially in the areas of Africa and Asia who are the main users (Dzoyem et al. 2019). In Uganda; expensive synthetic drugs, ineffective drugs, inaccessible drugs and lack of access to veterinary services are the driver to use of EVP. The practice is becoming popular because of the need of decreasing residues of synthetic drugs and the demand for organic food products (Busari et al. 2021). Reducing overuse of antimicrobials which are implicated in escalation of anthelmintic resistance (Bamidele et al. 2022).

Ethnoveterinary practices are a very important alternative in the era of organic farming and emerging anthelmintic resistance against the available synthetic anthelmintics. In this study we analyse the phytochemical composition of the two most ranked anthelmintic plants.

5.2 Material and methods

(Described in section **3.5.2** of chapter 3)

5.3 Results

Capsicum annuum L. & *Carica papaya L.* showed presence of alkaloid salts, coumarins, flavonosides and steroid glycosides. Only ethanoic extracts of *Capsicum annuum L.* showed presence of anthrocyanosides and anthraconosides. Only ethanol extracts of *Carica papaya L.* showed presence of saponins. None of the extracts showed presence of tannins (**Table 5**)

Table 5: Qualitative Phytochemical profile of *Carica papaya* L. and *Capsicum annuum* L. extracts

Phytochemical	*Carica papaya* L. extracts		*Capsicum annuum* L.	
	Ethanolic	Acetone	Ethanol	Acetone
Saponins	+	-	-	-
Tannins	-	-	-	-
Reducing compounds	+	-	+	-
Alkaloid salts	+	+	+	+
Anthocyanosides	-	-	+	-
Anthraconosides	-	-	+	-
Coumarins	+	+	+	+
Flavonosides	+	+	+	+
Steroid glycosides	+	+	+	+

+ Present - Absent

5.3.1 GC-MS profile of *Carica papaya* L. and *Capsicum annuum* L. extract

The acetone extract of *Carica papaya* L. leaves (CPL) yielded sterols (13%), vitamin C (42%) and triterpenoids (6%) (**Table 6**).The ethanolic extract of CPL yielded pyranones (20.3%), phenolics (3.1%), glycosides (2.2%), diterpenoids (4.9%), sterols (33%), triterpenoids (3.5%) and steroids (1.4%) (**Table 7**).The acetone extract of *Capsicum annuum* L. fruits (CAF) yielded sterols (45.04%), alkanes (27.7%) and alkaloids (8.2%) (**Table 8**).The ethanolic extracts of CAF yielded glycosides (3.61%), sterols (50.16%), pyranones (3.55%) and alkaloids (22.73%) (**Table 9**).

Table 6: GC-MS profile of *Carica papaya* L. leaves acetone extract

Retention time	Compound Name (AP)/ Group of compounds	CAS#	Formula	Component area	Match factor %	Estimated conc. (%)
4.1974	2H-Benzo[f]oxirenol[2,3-E] benzofuran-8(9H)-one,9-[[[2-(dimethylamino) ethyl] amino] methyl] octahydro-2,5a-dimethyl-	1000316-31-0	$C_{19}H_{32}N_2O_3$	30902350.6	57	31
16.791	9-Hexadecenoic acid, 9-octadecenyl ester, (z, z)- / **(sterols)**	22393-98-2	$C_{34}H_{64}O_2$	7707685.4	63.3	7.7
20.2233	1-(+)-Ascorbic acid 2,6-dihexadecanoate / **(vitamin C)**	28474-90-0	$C_{38}H_{68}O_8$	42045756.6	68.5	42
25.3152	2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-7,10-dihydroxy-1,1,36,9-pentamethyl-4a,7a-epoxy-5H-cyclopenta[a]cycloundecen-11-yl ester, [1aR-[1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*(E),11aS*]]- /**(sterols)**	51906-13-9	$C_{27}H_{38}O_8$	5309888.5	61.2	5.3
29.0563	D: A-Friedooleanan-3-ol, (3, alpha.)- / **(Triterpenoids)**	5085-72-3	$C_{30}H_{52}O$	6262628.8	51.2	6

**The acetone extract of *Carica papaya* L. leaves yielded 13% sterols and 6% triterpenoids which are some of the compounds with known anthelmintic action.

Table 7: GC-MS profile of *Carica papaya* L. leaves ethanolic extract

Retention time	Compound Name (EP)/ Group of compounds	CAS#	Formula	Component area	Match factor	Estimated conc. (%)
4.4683	Benzene,1,2- dichloro-	95-50-1	$C_6H_4Cl_2$	310372634.1	77.4	1.5
4.8495	Benzyl alcohol	100-51-6	C_7H_8O	656899632	74.3	3.1
5.5951	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- **/** (**Pyranones)**	28564-83-2	$C_6H_8O_4$	4244572882.5	79.7	20.3
7.0002	Phenol, 5-ethenyl-2methoxy- **/ (Phenolics**)	621-58-9	$C_9H_{10}O_2$	652974980.4	81.6	3.1
8.4054	Ethyl beta-d-riboside **/ (glycosides)**	1000126-95-4	$C_7H_{14}O_5$	470071693.6	70.8	2.2
16.7973	Neophytadiene /(**diterpenoids)**	504-96-1	$C_{20}H_{38}$	341714834.0	87.2	1.6
20.6459	n-Hexadecanoic acid **/ (sterols)**	57-10-3	$C_{16}H_{32}O_2$	1352846816.3	64.9	6.5
21.1578	Hexadecanoic acid, ethyl ester **/ (sterols)**	628-97-7	$C_{18}H_{36}O_2$	242270004.7	83.5	1.2
24.5382	Phytol /**(diterpenoids)**	150-86-7	$C_{20}H_{40}O$	699650422.6	88.3	3.3
25.6724	9,12,15-Octadecatrienoic acid (Z, Z, Z)- **/ (sterols)**	463-40-1	$C_{18}H_{30}O_2$	4364890955.8	87.4	20.8
26.4739	Octadecanoic acid **/ (sterols)**	57-11-4	$C_{18}H_{36}O_2$	639975153.6	84.3	3.1
36.3320	Supraene /(**Triterpenoids)**	7683-64-9	$C_{30}H_{50}$	731801424.0	84.5	3.5
43.8826	beta. -Sitosterol **/ (Steroids)**	83-46-5	$C_{29}H_{50}O$	284103679.4	81.2	1.4

The ethanolic extract of *Carica papaya* L. leaves yielded 25.3% sterols, 8.4% terpenoids and 3.1% phenolics which are some of the compounds with known anthelmintic action.

Table 8: GC-MS profile of *Capsicum annuum* L. fruits acetone extract

Retention time	Compound Name (AR)/ Group of compounds	CAS#	Formula	Component area	Match factor	Estimated conc. (%)
25.8299	9,12-Octadecadienoic acid (Z, Z)- /**(sterols)**	60-33-3	$C_{18}H_{32}O_2$	1344761417.7	88.4	9.91
26.0223	9,12,15-Octadecatrienoic acid, (Z, Z, Z)- **/(sterols)**	463-40-1	$C_{18}H_{30}O_2$	275995441.8	60.1	2.03
26.4819	Pentadecanoic acid **/(sterols)**	1002-84-2	$C_{15}H_{30}O_2$	546092918.2	83.6	4.02
32.5972	Heptacosane **/(alkanes)**	593-49-7	$C_{27}H_{56}$	1745180778	81.2	12.86
33.4436	Capsaicin /**(Alkaloid)**	404-86-4	$C_{18}H_{27}NO_3$	802053360.9	84.9	5.91
33.6958	Dihydocapsaicin **/(Alkaloids)**	19408-84-5	$C_{18}H_{29}NO_3$	311451357.4	72.5	2.29
34.9720	Heneicosane **/(alkane)**	629-94-7	$C_{21}H_{44}$	748932682.8	84.2	5.52
36.3398	Squalene **/(sterols)**	111-02-4	$C_{30}H_{50}$	3032372293.2	85.0	22.34
36.8537	1-Heptacosanol **/(sterol-alcohol)**	2004-39-9	$C_{27}H_{56}O$	384232719.6	85.3	2.83
38.1879	Hentriacontane / **(alkane)**	630-04-6	$C_{31}H_{64}$	1264907160	76.5	9.32
39.3970	1-Heptacosanol **/(sterols)**	2004-39-9	$C_{27}H_{56}O$	252395509.5	83.7	1.86
41.2582	Tetratetracontane **/(sterols)**	7098-22-8	$C_{44}H_{90}$	278416640.1	71.4	2.05

The acetone extract of *Capsicum annuum* L. fruits yielded 45.04% sterols and 8.2% alkaloids which are some of the compounds with known anthelmintic action

Table 9: GC-MS profile of *Capsicum annuum* L. ethanolic extract

Retention time	Compound Name (ER)/ Group of compounds	CAS#	Formula	Component area	Match factor	Estimated conc. (%)
6.2338	5-Hydroxymethylfurfural **/(glycosides)**	67-47-0	$C_6H_6O_3$	1039557851.9	81.7	3.61
16.1489	Oleic acid **/(sterol)**	112-80-1	$C_{18}H_{34}O_2$	1263336730	68.9	4.39
16.6178	Pentadecanoic acid **/(sterol)**	1002-84-2	$C_{15}H_{30}O_2$	806683607.3	86.5	2.80
18.8356	Palmitoleic acid **/(sterol)**	373-49-9	$C_{16}H_{30}O_2$	1194629505	84.0	4.15
19.2672	n-Hexadecanoic acid **/(sterol)**	57-10-3	$C_{16}H_{32}O_2$	1090877057	73.3	3.79
21.6884	Cic-10-Heptadecenoic acid /**(sterol)**	29743-97-3	$C_{17}H_{32}O_2$	602677070.9	81.7	2.09
26.0962	Ethanol, 2-(9,12-octadecadienyloxy)- (Z, Z)-	17367-08-7	$C_{20}H_{38}O_2$	1539700382	80.6	5.35
26.3316	2H-Pyran-2-one, tetrahydro-6-tridecyl- **/ (pyranones)**	1227-51-6	$C_{18}H_{34}O_2$	1021059290.5	55.2	3.55
26.4343	9,12,15-Octadecatrienoic acid, (Z, Z, Z)- /(**sterol)**	463-40-1	$C_{18}H_{30}O_2$	2107179713.2	67.2	7.32
26.8005	Octadecanoic acid **/(sterol)**	57-11-4	$C_{18}H_{36}O_2$	942150689.0	83.3	3.27
28.4168	Arachidamide, N-methyl- **/(sterol)**	1000420-44-0	$C_{21}H_{43}NO$	6433662652.3	64.0	22.35
31.4010	Capsaicin **/(Alkaloid)**	404-86-4	$C_{18}H_{27}NO_3$	4834637352	65.6	16.80
33.9070	Dihydrocapsaicin **/(Alkaloid)**	19408-84-5	$C_{18}H_{29}NO_3$	1706326041	78.9	5.93

The ethanolic extract of *Capsicum annuum* L. fruits yielded 50.09% sterols and 22.73% alkaloids which are some of the compounds with known anthelmintic action.

5.4 Discussions

5.4.1 Qualitative Phytochemical profile of *Carica papaya* L. and *Capsicum annuum* L

Saponins have been reported to have anthelmintic effects (Maestrini et al., 2020; Santos et al., 2018). Alkaloids are also reported to have action against helminths (Dubois et al. 2019; Rocha et al. 2017). Coumarins have inhibitory effects against helminths (Basumatary et al. 2020; Liu et al. 2021). Some flavonoids are effective against helminths (Azando et al. 2011; Zarzar-Albarran et al. 2020. Some steroids are effective against helminths (Whiteland et al. 2018).

5.4.2 Quantitative Phytochemical analysis of *Carica papaya* L. and *Capsicum annuum* L.

Ethanolic extracts of *Carica papaya* and *Capsicum annuum* showed a wider range of compounds than their acetone extracts. This relates to the claims of anthelmintic action by chicken owners who soak the crushed plant materials in water which is a polar solvent. A polar (ethanol) and a moderately polar solvent were selected for that reason. Steroids are said to have anthelmintic effects (Wang et al. 2010) but the action all specific lipids on helminths are not fully known. Vitamin C doesn't have particular action against helminths but it improves the response of hosts to helminths infections (Ince et al 2010). Triterpenoids have known effects on helminths (Chama et al. 2020; Kuzminac et al. 2023), however the specific effect of D: A-Friedooleanan-3-ol, (3, alpha.)-and Supraene against helminths requires further investigation. The action of pyranones against helminths are not known and requires further investigation. Phenolics have known action against helminths (Mukherjee et al. 2016; Ndjonka et al. 2014), however the particular effect of Phenol, 5-ethenyl-2methoxy- on helminths is not known. Particular glycosides especially flavonal glycosides have anthelmintic action (Kozan & Anul, 2013) however the action of all other types of glycosides against helminths require further research. Diterpenoids have known action against helminths (Crusco et al. 2018). Phytol is known to be effective against helminths (de Moraes et al. 2014), but the effect of Neophytadiene on helminths requires further research. The action of alkanes against helminths are not known and require further research. Alkaloids have known action against helminths (Dubois et al. 2019; da Silva et al.

2021); Capsaicin and Dihydocapsaicin are known to be effective against helminths especially due to their pungency action (Coronel et al. 2022). Not all the observed phyto-active substances were similar to those known to have anthelmintic action according to literature. Although the action of pyranones and alkanes against helminths were unknown, this wasn't considered as sufficient ground, I rejected the null hypothesis.

5.4.3 Conclusions and Future work

Capsicum annuum L. contained high concentrations sterols and alkaloids while *Carica papaya* L. contained sterols, terpenoids and phenolics. Although the observed phyto-compounds are said to have anthelmintic action experiments on the extracts in chicken is required to empirically validate the claims. Pyranones were in relatively high concentrations in both *Capsicum annuum* L. and *Carica papaya* L. although their anthelmintic action is unknown. Experiments of the effects of the plant candidates on helminths can provide an opportunity against the emerging resistance against synthetic anthelmintics. Single plant alternatives, plant combinations or plant-synthetic anthelmintic combinations can be used to generate data to advise the food and drug developing authorities.

CHAPTER SIX

IN-VITRO AND *IN-VIVO* EFFICACY OF *CASPSCUM ANNUUM* L. AND *CARICA PAPAYA* L. CRUDE EXTRACTS AGAINST CHICKEN HELMINTHS

6.1 Introduction

Chicken is a major economic resource in Soroti district which would be greatly improved with better health management (Kugonza et al., 2004). Local birds in Uganda usually free range and scavenge for food (Mwesigwa et al., 2015), such a management system predisposes chicken to many parasites.

Clinical helminths infestations causes unthriftness, diarrhoea, innappetence and stuntedness among chicken (Phiri et al., 2007; Robel et al., 2003; Van et al., 2020b). *Syngamus trachea* causes respiratory distress. *Heterakis gallinarum* hosts *Histomonas meleagridis* which causes histomoniasis (typhllo-hepatitis) (Papini & Cacciuttolo, 2008). *Ascaridia galli* are very large nematodes that lead to intestinal obstructions especially in chicks. Helminths lower chicken vaccine responses and can affect the general immune system of chicken.

The allopathic drugs used to control helminths include albendazole, levamisole, piperazine, pyrantel, ivermectin, nitarsone, tetramisole, phenothiazine, methyridine, coumaphos, haloxon and thiabendazole (Macklin & Hauck, 2019). The EVP include use of certain plant products or any other non-allopathic agents. The study purposed to evaluate *in-vitro* and *in-vivo* anthelmintic efficacy of CA and CP which were the top ranked ethno-anthelmintic materials in the earlier survey. The study was done after knowing the phytochemical profile of the two plant candidates.

6.2 Materials and Methods

(Described in section 3.5.3 of chapter 3)

6.3 Results

6.3.1 *In-vitro* Efficacy

The antihelmintic activity of extracts increased with time and was dose dependent.

Capsicum annuum L. extracts (CAFa & CAFe) took long to act as compared to piperazine citrate but showed anthelmintic activity. The extracts didn't cause any observable lesions on the skin whatsoever. Four to five hours was needed for the extracts to make *Ascaridia galli* paralysed. A concentration of 0.08g/ ml made over 5/10 of the mature *A. galli* paralysed after 5 hours while 0.32g/mL made over 8/10 mature *A. galli* paralysed. CPLe acted on *A. galli* faster than *Capsicum annuum* L. fruit extracts. After 240 min 0.32g/ml CPLe made 7/10 of adult *A. galli* paralysed. After 270 min, 0.08g/ml had made 5/10 of adult *A. galli* paralysed and by 300min, 0.08g/ml had made 6/10 of the adult *A. galli* paralysed. CPLa by 240min, 0.08g/ml had made 5/10 of adult *A. galli* paralysed and 0.32g/ml had made 10/10 of adult *A. galli* paralysed. By 300min, 0.08g/ml had made 7/10 of adult *A. galli* paralysed (Table 10).

Table 10: *In-vitro* efficacy tests of the extracts, positive control (Piperazine citrate) and negative control (PBS)

Treatment	Concentration g/mL		60min	90min	120min	150min	180min	210min	240min	270min	300min
		0.32	0.0±0.0	0.00±0.000	0.00±0.000	1.33±0.577	3.67±0.577	4.67±0.577	5.33±0.577	7.67±0.577	8.67±0.577
		0.16	0.0±0.0	0.00±0.000	0.00±0.000	0.33±0.577	3.00±1.000	3.67±0.577	4.67±0.577	5.67±0.577	7.33±0.577
CAFe		0.08	0.0±0.0	0.00±0.000	0.00±0.000	0.00±0.000	2.67±0.577	3.00±1.000	3.33±1.528	3.67±1.155	5.33±0.577
		0.04	0.0±0.0	0.00±0.000	0.00±0.000	0.00±0.000	1.33±0.577	1.67±0.577	1.67±0.577	2.67±0.577	4.33±0.577
		0.02	0.0±0.0	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.33±0.577	1.00±0.000	1.33±0.577	2.67±0.577
		0.32	0.0±0.0	1.33±1.155	2.33±1.155	5.33±0.577	6.33±0.577	7.33±0.577	8.33±0.577	9.00±0.000	9.67±0.577
		0.16	0.0±0.0	0.33±0.577	0.67±1.155	4.00±1.000	4.33±0.577	5.67±1.155	6.33±0.577	6.67±1.155	9.00±1.000
CAFa		0.08	0.0±0.0	0.00±0.000	0.33±0.577	2.67±1.155	3.00±1.000	4.67±0.577	5.00±1.000	5.33±0.577	6.67±0.577
		0.04	0.0±0.0	0.00±0.000	0.00±0.000	0.67±1.155	1.67±1.528	3.33±0.577	4.00±1.000	4.33±0.577	5.00±1.000
		0.02	0.0±0.0	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.67±0.577	1.00±0.000	1.33±0.577	1.67±0.577
		0.32	0.0±0.0	0.00±0.000	1.67±1.155	2.67±1.155	5.00±1.000	7.00±0.000	7.67±0.577	8.67±0.577	9.33±0.577
		0.16	0.0±0.0	0.00±0.000	1.00±1.000	2.33±1.528	4.67±0.577	5.33±0.577	6.33±0.577	6.67±0.577	7.33±0.577
CPLe		0.08	0.0±0.0	0.00±0.000	0.33±0.577	0.67±0.577	2.33±1.155	3.67±0.577	4.33±0.577	5.33±0.577	7.00±1.000
		0.04	0.0±0.0	0.00±0.000	0.00±0.000	0.67±0.577	2.33±1.155	2.67±1.528	3.67±1.155	4.00±1.000	5.33±0.577
		0.02	0.0±0.0	0.00±0.000	0.00±0.000	0.00±0.000	0.33±0.577	0.67±0.577	2.00±1.000	2.67±0.577	3.67±0.577
		0.32	0.0±0.0	0.00±0.000	3.67±0.577	5.00±1.000	6.00±0.000	9.33±0.577	10±0.000	10.0±0.000	10.0±0.000
		0.16	0.0±0.0	0.00±0.000	1.67±0.577	4.33±0.577	5.33±0.577	5.67±0.577	7.33±0.577	8.67±0.577	8.67±0.577
CPLa		0.08	0.0±0.0	0.00±0.000	1.33±0.577	1.67±1.155	3.67±0.577	4.67±0.577	5.67±0.577	7.33±0.577	7.33±0.577
		0.04	0.0±0.0	0.00±0.000	0.00±0.000	0.33±0.577	2.67±0.577	3.33±0.577	4.33±0.577	5.67±0.577	5.67±0.577
		0.02	0.0±0.0	0.00±0.000	0.00±0.000	0.00±0.000	0.33±0.577	1.00±1.000	1.33±1.155	2.33±1.155	2.67±0.577

Pip-citrate	0.025	1.0±1.0	3.00±1.000	8.33±1.528	9.33±0.577	10.0±0.000	10.0±0.000	10.0±0.000	10.0±0.000	10.0±0.000
PBS		0.0±0.0	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000

Key: *Carica papaya* leaves ethanol extract (CPLe), *Carica papaya* leaves acetone extract (CPLa), *Capsicum annuum* ripe fruits ethanol extract (CAFe), *Capsicum annuum* ripe fruits acetone extract (CAFa) and phosphate buffered saline (PBS)

6.3.2 *In-vivo* efficacy test of crude extracts of *Capsicum annuum* L. and *Carica papaya* L

All extracts were significantly more effective compared to PBS (p =0.000), all extracts caused higher FECR than piperazine citrate *(p=0.000)* and CPLa was as good as levamisole *(p=0.993).* Although CAFa & CAFe were effective but CPLa was the most effective, almost as good as levamisole. **(Table 11& Appendix (vii))**

Table 11: *In-vivo* Efficacy tests of the extracts, Piperazine and Levamisole

No.	Code	ECG_1	ECG_2	ECG_3	$FECR_1$ (%)	$FECR_2$ (%)	$FECR_3$ (%)	Mean ±SD FECR (%)
1	CAFa	2900	2805	2493	80	78	81	79.67±1.528
2	CAFe	3250	3188	2886	76	75	78	76.33±1.528
3	CPLa	0	638	262	100	95	98	97.67±2.517
4	CPLe	6100	5865	6298	56	54	52	54.00±2.000
5	PiP	9000	7905	8659	35	38	34	35.67±2.082
6	Lev	0	510	0	100	96	100	98.67±2.309
7	PBS	13800	12750	13120	0	0	0	0.00±0.000

Key: Egg counts per gram of faeces (ECG), faecal egg count reductions (FECR), *Carica papaya* leaves ethanol extract (CPLe), *Carica papaya* leaves acetone extract (CPLa), *Capsicum annuum* ripe fruits ethanol extract (CAFe), *Capsicum annuum* ripe fruits acetone extract (CAFa), levamisole hydrochloride (Lev), Piperazine citrate (Pip) and phosphate buffered saline (PBS)

6.4 Discussion

6.4.1 *In-vitro* Efficacy tests of the extracts

No deaths were observed nor any observable lesions on the *A. galli* dermis, however the extracts inhibited motility with no change of colour of worms. The extracts were significantly effective compared to PBS but acted slowly compared to piperazine citrate. The action of *Carica papaya* L. extracts is in agreement with the findings of Nghonjuyi et al., (2020) and Sugiharto, (2020). The findings regarding *Capsicum annuum* L. extracts are in agreement with Gentiles et al. (2019) who reported the high anthelmintic potency of *Capsicum annuum* var. Longum. The CPLa was faster than CPLeAll *Carica papaya* L. extracts had terpenoids whose action against nematodes is said to be boosted by vitamin C (Rashed et al., 2024) . The findings are in agreement

with Sen et al. (2020) who found 100% *in-vitro* effect against *A. galli* at even 20mg/ml, he also showed that the extracts were slower and required longer time periods in terms of five to seven hours. The findings are also in agreement with Cabral et al., (2019) who achieved 100% *in-vitro* action against *Strongyloides stercoralis* using 566mg/ml of *Carica papaya* L. extracts. Increasing the concentration several folds reduced the action time in the *in-vitro* assays.

6.4.2 *In-vivo* Efficacy tests of the extracts, Piperazine and Levamisole

All extracts were effective with above 50% faecal egg count reductions; however, the *Carica papaya* L. acetone extracts were more efficacious compared to all *Capsicum annuum* L. extracts (Table 6.2). The CPLa was distinctly more efficacious compared to CPLe in faecal egg reductions. The superior action was attributed to the vitamin C in presence of other anthelmintic compounds. The action of vitamin C on helminths in presence of various types of anthelmintics is not well known although Sengupta et al., (2023) mentions that the observed actions arise from vitamin C enhancing the body defence lines. The findings about the *in vivo* efficacy of *Carica papaya* L. extracts are in agreement with Sen et al., (2020) . The findings regarding the *in-vivo* action of *Capsicum annuum* L. extracts are in agreement with Gentiles et al., (2019) who observed significant faecal egg reductions.

CPLa was as effective as levamisole, unlike CPLe, CPLa had higher concentration of vitamin C. All extracts had higher FECR than piperazine citrate at its recommended therapeutic dose; this was in line with Kateregga et al., (2014) where the methanolic extract of *cassia occidentalis* L significantly exhibited higher mean *A.galli* mortality than piperazine citrate. Levamisole hydrochloride caused the highest faecal egg count reduction. The results show that there is no levamisole hydrochloride anthelmintic resistance in chicken in the study area although piperazine citrate anthelmintic resistance is likely. Chicken owners are advised to adopt the ethnoveterinary approaches in lieu of piperazine-citrate if levamisole hydrochloride is not accessible. Pyranones were in relatively high concentrations in both CAF and CPL, it's imperative to evaluate them for possible anthelmintic action. There is urgent need for comprehensive extract toxicity studies before they are purified and recommended for industrial scaling up. The role of vitamin C in anthelmintic actions requires further investigation to explore all opportunities in other herbal and synthetic combinations.

Reject the null hypothesis because there is enough evidence to support the alternative claim.

6.4.3 Conclusion and Future work

Pyranones were in relatively high concentrations in both CAF and CPL, it's imperative to evaluate them for possible anthelmintic action. There is urgent need for comprehensive extract toxicity studies before they are purified and recommended for industrial scaling up. The role of vitamin C in anthelmintic actions requires further investigation to explore all opportunities in other herbal and synthetic combinations.

CHAPTER SEVEN

COMPARING THE TOXICITY OF SELECTED PLANT EXTRACT ANTHELMINTICS TO LEVAMISOLE HYDROCHLORIDE AND PIPERAZINE CITRATE IN CHICKENS

7.1 Introduction

Synthetic and natural therapeutic products can both induce toxicities, especially when administered at very high doses (Abdel-Daim et al., 2018). Most (81%) of the plant extracts were reported to have toxic effects in various animal tissues, unless dosed prudently (Viegi & Vangelisti, 2011). Although many plants have been evaluated for their ethnoveterinary potential, few of them have been assessed for toxicity (Habeeb, 2010; McGaw & Eloff, 2008; Sunder, 2016).

Damage to the kidney of any cause manifests with changes in the concentrations of Blood Urea Nitrogen (BUN), creatinine, uric acid and changes in serum osmolality (Gounden et al., 2021; Kluwe, 1981). Hepatoxicity may manifest as hepatomegaly, presence of haemorrhages, focal necrosis, discolourations, accumulation of lipids in hepatocytes, jaundice, biliary tissue proliferation, focal fibrosis and hyperplasia (Sathiyanarayanan & Arulmozhi, 2007). Various plant extracts and commercial drugs have been shown to have toxic effects on the cardiovascular system of animals. Toxic substances may cause congestion of coronary vessels (Latif et al., 2024).

Haematological parameters are affected by animal physiological condition, genotype, age, sex, nutrition, climatic conditions and prevailing pathology. There is no information on haematology of indigenous chicken of Uganda.

This study intended to compare the toxicological indices of ethno-anthelmintics (*Capsicum annuum* L. and *Carica papaya* L.) to the commonly used synthetic anthelmintics (piperazine citrate and levamisole hydrochloride). The indices considered here are haematology, renal functions, liver functions and selected organ histopathology.

7.2 Materials and Methods

(Described in section **3.5.4** of chapter 3)

7.3 Results

7.3.1 Observation for adverse events

No lethargy, diarrhoea, dropping saliva or ruffled feathers were observed and no deaths were recorded in any of the treatment groups.

7.3.2 Renal function parameters

The results of blood urea nitrogen, sodium, chloride, creatinine and uric acid measurements are shown in Table 12. There was no difference in the effects on renal parameters between the plant extracts (CPLa, CPLe, CAFa & CAFe) and the synthetic anthelmentics. However, CAFa extract demonstrated significantly lower levels of sodium compared to CPLe extract ($P = 0.046$). CAFe and piperazine citrate increased sodium level compared to CAFa ($p = 0.005$, $p = 0.04$), respectively.

Table 12: Renal function parameters in chickens treated with herbal and synthetic anthelmintics

#	Plant extracts	BUN mmol/L	Na mmol/L	Cl mmol/L	Cre µmol/L	Uric acid (mmol/L)
1	CAFa	7.118±4.36	**15.363±0.55[a]**	97.733±2.25	0.860±0.11	0.100±0.05
2	CAFe	10.567±2.16	27.967±2.38[c]	94.433±1.93	1.250±0.16	0.178±0.04
3	CPLa	3.413±5.61	24.213±3.83	92.667±2.87	1.177±0.35	0.106±0.04
4	CPLe	5.767±3.23	**24.583±4.20[b]**	95.167±4.10	0.700±0.17	0.127±0.03
5	Pip	5.197±2.07	**24.774±1.25[c]**	94.533±1.08	0.853±0.28	0.161±0.13
6	Lev	5.710±3.83	34.343±2.02	96.833±1,51	0.843±0.13	0.112±0.06
7	PBS	5.370±4.05	23.440±5.51	94.300±1.65	0.837±0.22	0.138±0.05

Key: BUN-blood urea, Na-sodium, Cl-chloride, Cr-creatinine.*a, b, c they are significantly different along the colunms. *Carica papaya* leaves ethanol extract (CPLe), *Carica papaya* leaves acetone extract (CPLa), *Capsicum annuum* ripe fruits ethanol extract (CAFe), *Capsicum annuum* ripe fruits acetone extract (CAFa), levamisole (lev), Piperazine (pip) and phosphate buffered saline (PBS)

7.3.3 Liver function parameters

The results of the liver function parameters are shown in Table 13. There was no significant difference in the effects on liver function parameters for all the treatment groups compared to the control ($p > 0.05$). However, CPLe demonstratrated significantly high albumin ($p = 0.02$) and levamisole hydrochloride demonstratrated significantly high serum AST ($p = 0.04$) compared to CAFe.

Table 13: Liver function parameters in chickens administered with herbal and synthetic anthelmintics

Treatment	AST (IU/L	ALT (IU/L)	ALP (IU/L)	GT (IU/L)	TB (µmoles/L)	DB (mm/L)	TP (g/L)	ALBU (g/L)	Globu (g/L)
CAFa	263.167±21.4	3.0±0.3	1330±335	23.33±1.5	0.30±0.10	0.17±0.06	49.63±5.51	13.633±0.72	36.0±5.7
CAFe	253.667±46.26	3.1±0.6	841.0±141.1	18.67±5.0	0.37±0.15	0.17±0.06	44.20±8.97	11.800±1.68	32.4±8.1
CPLa	276.333±49.64	3.1±0.8	873.7±700.2	19.67±3.8	0.23±0.15	0.00±0.00	47.03±8.47	13.233±1.38	33.6±7.3
CPLe	**324.867±49.79[b]**	2.8±0.4	1367±264	23.67±3.5	0.23±0.06	0.10±0.10	47.00±7.85	**15.733±0.71[a]**	31.3±7.8
Pip	268.567±8.81	2.5±0.9	1810±1447	27.33±6.5	0.30±0.20	0.10±0.10	42.60±1.48	14.733±1.30	27.9±2.7
Lev	357.167±6.88	3.7±0.1	620.3±79.5	17.33±3.1	0.43±0.12	0.20±0.10	50.40±2.27	12.633±0.51	39.3±2.4
PBS	261.267±36.60	2.7±0.4	959.3±313	20.33±5.0	0.13±0.12	0.07±0.12	48.07±1.46	15.100±1.71	33.0±3.1

Key: AST (Aspartate aminotransferase), ALT (Alanine transaminase), ALP (Alkaline phosphatase), GT (Glutamyl transferase), TB (Total bilirubin), DB (Direct bilirubin), TP (Total protein), ALBU (Albumin), Globu (Globulins). Mean of the parameters ±standard deviations.

***a, b** they are significantly different along the colunms. *Carica papaya* leaves ethanol extract (CPLe), *Carica papaya* leaves acetone extract (CPLa), *Capsicum annuum* ripe fruits ethanol extract (CAFe), *Capsicum annuum* ripe fruits acetone extract (CAFa), levamisole (lev), Piperazine (Pip) and phosphate buffered saline (PBS)

7.3.4 Haematology

The results of the haematological parameters are shown in **Table 14**.

There were no significant differences in the haematological parameters when comparing chickens treated with CPLa, CAFa, CPLe, CAFe, piperazine citrate, levamisole hydrochloride to the PBS group, except for eosinophils and monocytes. There was no significant difference in haematological parameters when comparing chickens that were treated with plant extracts (CPLa, CPLe, CAFa & CAFe) to those that were treated with synthetic anthelmintics (piperazine citrate and levamisole hydrochloride) (*P>0.05*), (**Appendix x**). CAFa and Piperazine treatment groups showed significantly lower eosinophil numbers than the control group, (*p=0.000*) for all. CAFe, CPLa, and Levamisole treatment groups showed a significantly increased eosinophils levels than control group *(p=0.01, p=0.017 & p=0.001*) respectively.

Levamisole group showed significantly more monocytes than the control group *(p=0.009)*, (**Appendix x**).

Table 14: Haematological parameters from chickens on herbal and synthetic anthelmintics

Treatment	PCV %	TPP g/dL	FIB g/dL	TWBC uL	TRBC uL	HB g/dL	EOS %	MON %	LYM %	HTR %	BAND %
CAFa	**26.17±2.93**	3.20±0.36	0.23±0.06	9.07±1.32	3.27±0.98	7.23±0.38	**2.00±1.00**[a]	4.67±3.06	39.67±13.80	53.00±15.72	4.00±1.0
CAFe	30.50±4.09	4.13±0.11	0.13±0.06	10.63±4.41	4.03±0.51	6.67±1.17	**3.00±1.00**[c]	3.67±1.53	39.33±9.24	51.00±8.54	5.67±4.5
CPLa	25.67±4.04	3.17±0.21	0.10±0.00	5.73±2.87	2.83±0.25	6.33±1.55	**5.00±1.00**[c]	8.00±3.61	61.67±18.56	27.33±17.01	4.33±1.5
CPLe	28.67±4.93	3.03±1.01	0.10±0.00	10.1±11.02	4.00±1.83	7.93±2.01	5.00±2.65	7.67±1.53	48.33±17.50	44.7±23.0	2.00±1.0
Pip	**30.00±1.00**	3.57±0.25	0.20±0.10	7.23±1.94	4.13±0.91	8.00±0.40	**2.00±1.00**[a]	10.00±2.65	38.00±5.29	50.33±2.52	2.33±0.5
Lev	25.50±2.50	3.95±0.35	0.15±0.05	5.05±1.95	3.15±0.85	6.60±0.60	**10.00±2.00**[c]	**16.00±6.00**[c]	35.00±1.00	34.00±4.0	5.00±1.0
PBS	29.33±2.08	3.30±0.26	0.13±0.06	7.70±4.12	3.67±1.20	7.17±1.04	**2.67±1.16**[b]	**4.33±2.08**[b]	46.67±9.02	50.00±9.17	5.00±2.0

Key: PCV (Packed cell volume), TPP (Total plasma proteins), FIB (Fibrinogen), TWBC (Total white blood cells), TRBC (Total red blood cells), HB (Haemoglobin), EOS (Eosinophils), MON (Monocytes), LYM (Lymphocytes), HTR (Heterophils). All the treatments were done in triplicates.

Mean of the blood parameters±standard deviations. *a, b, c they are significantly different along the colunms *Carica papaya* leaves ethanol extract (CPLe), *Carica papaya* leaves acetone extract (CPLa), *Capsicum annuum* ripe fruits ethanol extract (CAFe), *Capsicum annuum* ripe fruits acetone extract (CAFa), levamisole (lev), Piperazine (Pip) and phosphate buffered saline (PBS)

7.3.5 Organ histopathology

7.3.5.1 Heart

For all treatments had no histopathologic effect on the heart tissues as shown in **Figure 3**.

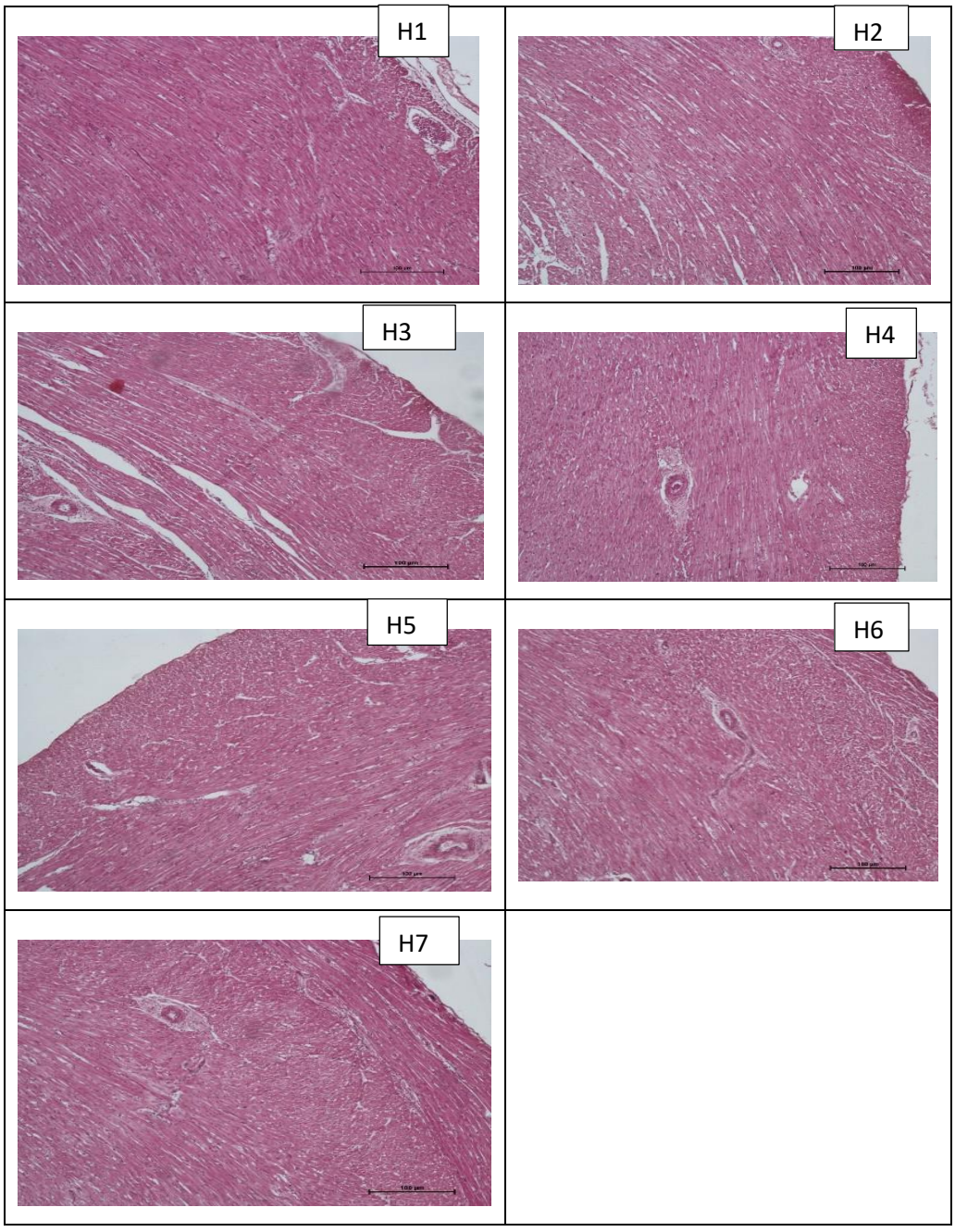

Figure 3: Histopathology micrographs of heart sections from chickens treated with plant extracts and synthetic anthelmintics (H&E stain) at 200^{X};

Key: (H1) CAFa treated heart, (H2) CAFe, (H3) CPLa, (H4) CPLe, (H5) piperazine citrate, (H6) levamisole HCl, (H7) PBS (control). There were no lesions in the heart in all treatment groups.

7.3.5.2 Kidneys

CAFa and Levamisole hydrochloride caused observable kidney lesions in (1/3) of the chicken, CAFe caused observable kidney lesions in all the three chicken (3/3), CPLa caused observable kidney lesions (2/3) of the chicken, CPLe, piperazine citrate, and PBS didn't cause any observable lesions in any of the three chickens (**Figure 4)**.

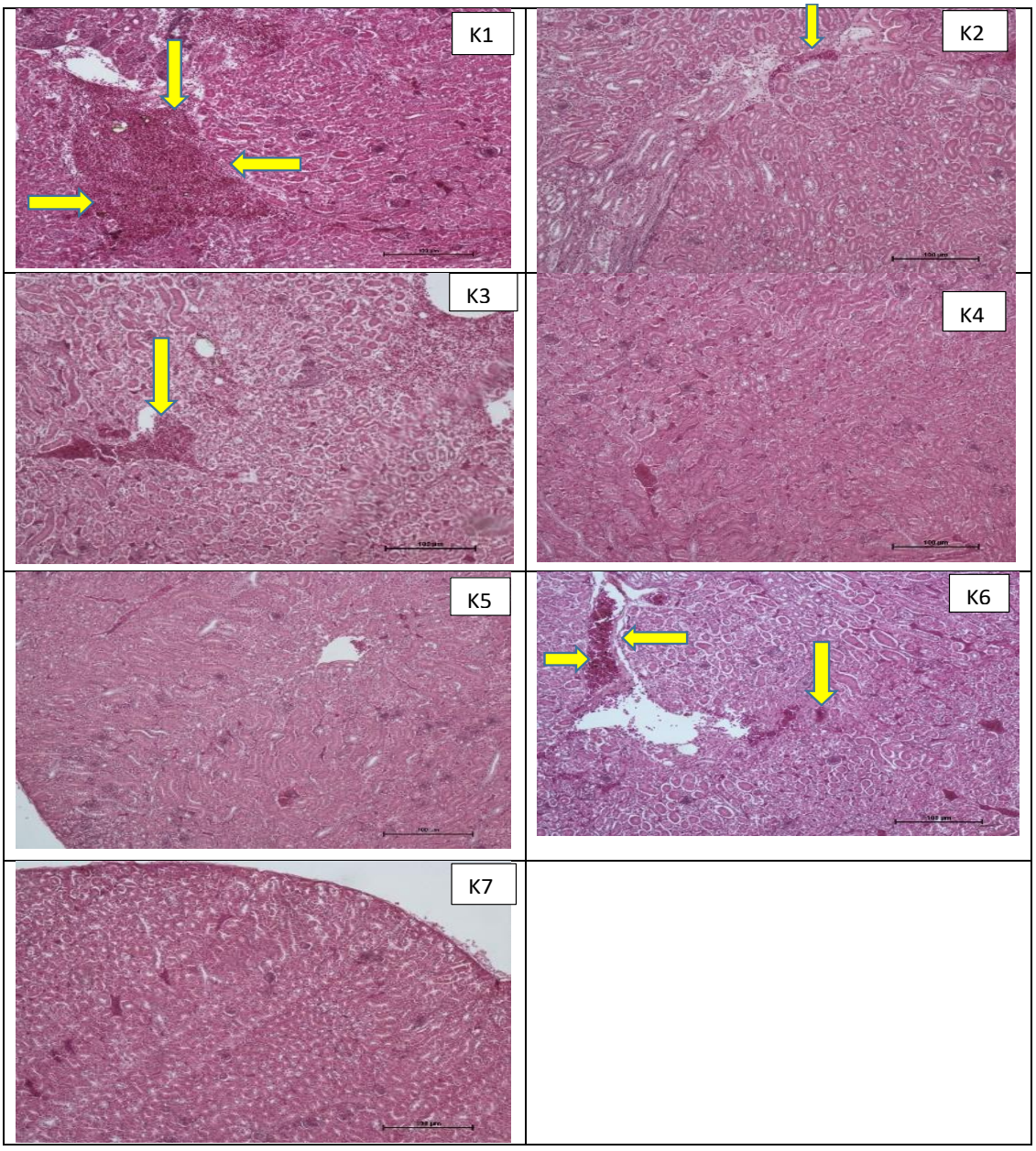

Figure 4: Histopathology micrographs of kidney sections from chickens treated with plant extracts and synthetic anthelmintics, at 200^{X}

Key: (K1) CAFa (Wide haemorrhages in renal cortex and medulla), (K2) CAFe (Renal haemorrhages and interstitial nephritis), (K3) CPLa (Renal congestion and haemorrhages), (K4), CPLe (No observable lesions), (K5) piperazine citrate (No observable lesions), (K6) levamisole HCl (Renal congestion and haemorrhages), (K7) (No observable lesions).

7.3.5.3 Liver

CAFa, CAFe, CPLa, piperazine citrate and levamisole hydrochloride caused observable liver lesions in (1/3) chickens, whereas CPLe and PBS didn't cause any observable liver lesions in any of the three chickens (0/3) **Figure 5**.

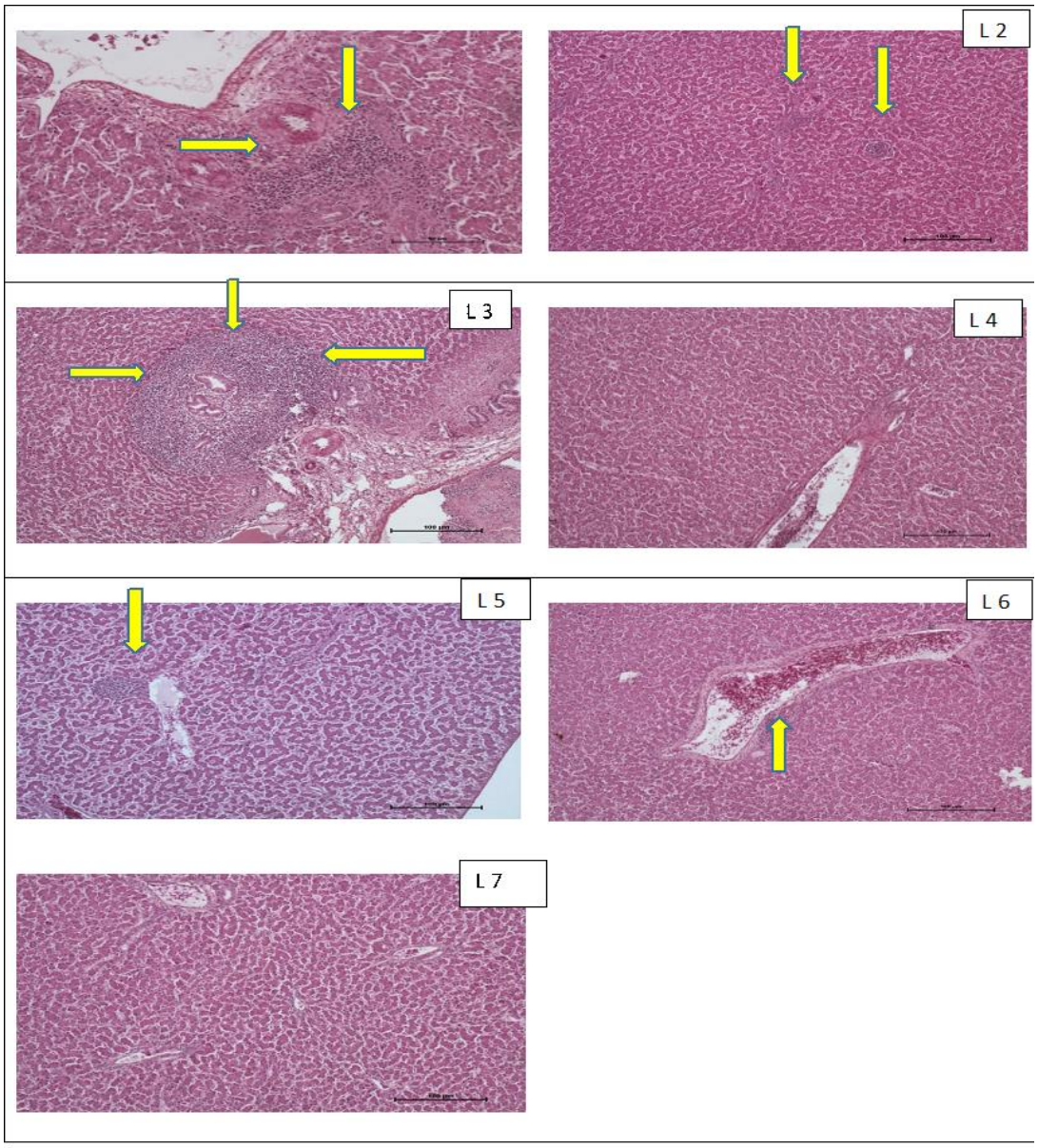

Figure 5: Histopathology micrograph of liver sections from chickens treated with plant extracts and synthetic anthelmintics at 200^{X}

Key: (L1) Liver of chicken treated with CAFa (Severe periportal necrotising hepatitis), (L2) Liver of chicken treated with CAFe (Multi-focal hepatitis), (L3) Liver of chicken treated with CPLa (Severe wide spread periportal necrotising hepatitis), (L4) Liver of chicken treated with CPLe (No observable lesions), (L5) Liver of chicken treated with piperazine (Periportal hepatitis), (L6) Liver of chicken treated with levamisole (Moderate periportal hepatitis), (L7) Liver of chicken treated with PBS (No observable lesions).

The summary of organ histopathology findings is shown in Table 15.

The order of causing histopathological lesions was CAFe>CPLa>CAFa. The extracts were safe to the heart but caused inflammations of kidney and liver except CPLe that didn't cause any lesions.

Table 15: The summary of organ histopathology findings

Treatment	Heart lesions	Kidney lesions	Liver lesions	Safety score
PBS	0/3	0/3	0/3	0/9
CAFa	0/3	1/3	1/3	2/9
CAFe	0/3	3/3	1/3	4/9
CPLa	0/3	2/3	1/3	3/9
CPLe	0/3	0/3	0/3	0/9
Piperazine citrate	0/3	0/3	1/9	1/9
Levamisole	0/3	1/3	1/3	2/9

7.4 Discussion

The results were analysed by linear regressions followed by Tukey's Honestly Significant Difference test. CAFa, CAFe, CPLa and CPLe, when compared to PBS, did not significantly affect renal function parameters, however, CAFa needs to be used with caution because it led to lower sodium in blood. The extracts didn't show significant effects on the electrolyte balance with regard to BUN, sodium, chloride, creatinine and uric acid. Reading renal functions, the extracts are safe for use at the given concentrations. Although the extracts didn't affect the sodium electrolyte balance, CAFa caused minor lowering of blood sodium compared to other extracts and piperazine citrate. Lower blood sodium predisposes to decreased blood pressure, increased uric acid, increased haematocrit, shock and heart failure (Julian, 1987; Sad, 2016). The plant extracts were as safe as the synthetic anthelmentics regarding effects on the BUN, sodium, Chloride, creatinine and uric acid balance. The extracts as well as piperazine citrate and levamisole hydrochloride had no effects on the metabolic actions that yields uric acid. The treatments didn't affect the glucose transporter 9 which regulates serum uric acid in chicken (Ding et al., 2021). The extracts didn't significantly

affect the liver functions, chronic toxicity studies are required to confirm the findings. However, CPLe caused more albumin compared to CAFe implying it led to more dehydration than CAFe. Levamisole hydrochloride increased AST compared to CAFe implying that levamisole chloride is more likely to cause liver damage compared to CAFe (Khanam et al. 2016).

At the therapeutic dose; the plant extracts (CAFe, CPLa & CPLe) and levamisole, caused increased blood eosinophils, although helminth infestations can also cause eosinophilia (Fuentebella et al. 2011; Maxwell, 1987) the findings point to more than that. The placebo with higher helminths loads had less blood eosinophils compared to the treatments possibly because the inflammatory action of the treatments was greater than action of helminths that leads to eosinophilia. Eosinophils are involved in the starting inflammatory responses, they can act as antigen presenting cells, are particularly responsible for defence against parasite infections and they modulate host's general immune responses (Rothenberg & Hogan, 2006). Eosinophilia is also observed in autoimmune disorders and endocrine disorders (Awad et al., 2023; Gigon et al., 2023; Kanuru & Sapra, 2023), however this has never been studied in chickens. Like levamisole; the extracts possibly induced hypersensitivity, autoimmune disorders or neoplasm defence mechanisms which manifested as eosinophilia, however the mechanisms driving this eosinophilia require further investigation.

The extracts of CAFa and piperazine caused eosinopenia because of the stress and response to the inflammatory reactions in the various organs. Eosinipenia is associated with stress and body response to inflammations (Zini, 2011). The monocytosis observed in levamisole hydrochloride treatments implies that it caused more stress and autoimmune disorders compared to the control. Monocytosis is observed in stressful conditions and during onset of autoimmune disorders (Taebipour et al., 2017). This is also because levamisole hydrochloride is an immunomodulator (Gholami et al., 2023). The plant extracts were as safe as piperazine citrate but safer than levamisole chloride regarding effects on the haematological parameters balance.

The different plant extracts, piperazine and levamisole did not show any observable lesions in the heart muscle sections of chickens. The anthelmintic extracts are not toxic to the heart muscles and major vessels. The findings relating to *Capsicum annuum* L. (acetone red pepper extract & ethanol red pepper extract) are in agreement with Mandal

et al., (2023) who reported that *Capsicum annuum* L. is cardio-protective. Sanati et al. (2017) also reported that *Capsicum annuum* L. has beneficial effects on the heart. The findings relating to *Carica papaya* L. (acetone pawpaw leaves extract & ethanol pawpaw leaves extract) are in agreement with Haramaki et al., (1995); Hasimun et al., (2020); Kong et al., (2021) who described the cardio-protective action of *Carica papaya* L.

Acetone red pepper extract (CAFa), ethanol red pepper extract (CAFe), and acetone pawpaw leaves extract (CPLa) were toxic to kidneys (Figure 2). The actions of *Capsicum annuum* L. are in agreement with Jabar & Jassim, (2023) who observed kidney histological alterations among animals treated with *Capsicum annuum* L.. Yuca, (2022) also reported kidney damages among animals that received very high doses of *Capsicum annuum* L. The mechanisms of CPLa toxicity are unknown because *Carica papaya* L. is reported in the literature as nephro-protective (Francis et al. 2020). It is possible that acetone yields some compounds with nepro-toxic effects compared to ethanol. However further investigations are needed to prove the variations in the compounds yielded with the different solvents and the renal effects of such compounds. Levamisole hydrochloride was equally toxic to the kidneys but piperazine citrate was safe. Nephro-toxic effects of levamisole have been reported in mice (Almawla & Al Baggou, 2023), however there are no such earlier reports in chicken. There is a need to confirm the findings regarding the safety of levamisole in chickens at the efficaciou doses.

All extracts were toxic to the liver except CPLe; equally piperazine and levamisole were toxic. The reports regarding *Capsicum annuum* L. are in disagreement with Das et al., (2018); Effendi & Sukmanadi, **(**2021); Oloruntola et al., (2024) who reported the hepato-protective actions of *Capsicum annuum* L, though in other species. *Carica papaya* L. is also reported to be hepato-protective as for CPLe (Awodele et al. 2016; Shaban et al. 2021). However, CPLa was toxic, possibly because of the difference in compound yields of acetone compared to ethanol. The hepatic findings of the effects of piperazine were in agreement with those of Bakhrebah et al. (2011), who observed histological changes in the chicken liver. The findings regarding levamisole were in agreement with Shovon et al, (2020), who reports that high doses of levamisole above 3mg/kg body weight are detrimental to the body. Generally, therefore, all plant extracts

were toxic except CPLe, levamisole was also toxic and piperazine was moderately toxic. CPLe was as safe as the placebo, safer than the two synthetic anthelmintics (piperazine and levamisole) that are commonly used by commercial chicken farmers in Uganda. I couldn't reject the the null hypothesis because the extracts were not safe just as piperazine citrate and levamisole were not safe at the therapeutic concentration.

7.4.1 Strength and Limitations

The experimental design set same cohort of chickens to triangulate the toxicity evaluation using the parameters of haematology, renal function tests, liver function tests and organ histotoxicity. This integrated assessment is used to derive a trustable conclusion. The concentrations of the extracts used were proven in previous efficacy tests, the concentration of piperazine citrate and levamisole hydrochloride were those recommended by the manufacturers and other researchers. PBS treatment is included as the placebo.

The study focusses on the toxic effects at the effective concentration of the extracts, the effects of extremely high doses was not studied. Immediate effects were not studied because samples were collected one week after the last treatment. The study doesn't consider the effects of chronic use of the treatments because chickens were not kept for a year or two years. Isolated or individual parasite effects as possible confounders of toxicity were not assessed.

7.4.2 Implications or recommendations

Capsicum annuum L. and *Carica papaya* L. are possible treatment alternatives to modern pharmaceuticals for chicken helminth infestation. The organ toxicity study showed that CPLe was the safest extract. Other extracts caused observable organ toxicity lesions in the order of CAFa < CPLa < CAFe. I recommend evaluating the toxicity of pure extracts such that the most effective fractions are scaled up as alternatives to synthetic anthelmintics. Toxicological assessment after prolonged use of the extracts should be evaluated with a larger sample size before recommending for wider use in chicken. The study should be repeated in different breeds of chicken in various geographical zones to identify any possible variations. The safety concerns of piperazine citrate and levamisole hydrochloride are critical requiring more studies for

confirmation and to make decisions on concentrations. Safety studies of highly purified extracts are recommended.

7.4.3 Conclusion

The extracts *Carica papaya* L. and *Capsicum annuum* L. didn't affect the hepato-renal function parameters, however most of them caused inflammations in kidneys and liver of the chickens. CAFa caused lowered number of eosinophils while CAFe, CPLa & CPLe caused increased number of eosinophils. Generally, there was no observed difference in safety between the plant anthelmintics and the synthetic anthelmentics (piperazine citrate & levamisole hydrochloride), all should be used with caution.

The study demonstrates the relative toxicity of piperazine citrate and levamisole hydrochloride at recommended concentrations compared to plant extracts used as anthelmintics in chicken.

CHAPTER EIGHT

GENERAL DISCUSSION

The study found that most farmers were able to state the signs that can be used to identify chicken infested with helminths. Some farmers (20%), were not deworming their chicken completely; such an inclination affects production and productivity manifesting as low weight gains and high FCR (Shifaw et al., 2021; Van et al., 2020a). The deworming regimes varied from one farmer to another usually not having the lifecycle of the helminths in consideration. Although the regime depends on the phytochemical type, its anthelmintic potency and abundance in the medicinal plant , most farmers'regimes were too long for helminths with 2-3 months lifecycle (Macklin & Hauck, 2019). Only about (16.7%) 13/78 were deworm after every three months, this would be the recommended based on the rational of the helminths lifecycles. About (32.1%) 25/78 were deworming after every one to two months, this is an overuse of anthelmintics which could lead to toxicity in chicken. Overuse of low doses of drugs with anthelmintic activity could also lead to development of resistance to anthelmintics (Fissiha & Kinde, 2021b). Anthelmintic resistance has already been reported in chicken in Uganda however it's not known whether herbal drugs have partly led to synthetic anthelmintic resistance.

The seven most cited anthelmintic plant species were in the discendng order; *Capsicum annuum* L. (Solanaceae), *Carica papaya* L. (Caricaceae), *Chemaecrista nigricans* (Vahl) Greene (Caesalphioideae), *Aloe barbadensis* Miller (Aloeacae), *Azadirachta indica* Juss. (Meliceae), *Warburgia ugandensis* Sprague subsp.ugandensis (Canelaceae) and *Portulaca quadrifida* L. (Portulacaceae). *Capsicum annuum L.* (Solanaceae) has phytochemicals with anthelmintic properties (Farooq et al., 2008). Various parts of *Carica papaya* L. (Caricaceae) are reported to contain compounds that can destroy helminths (Dotto & Abihudi, 2021). Extracts of *Chemaecrista nigricans* (Vahl) Green were reportedly effective against helminths (Osunga et al., 2023). Aloe extracts are said to have lethal action against helminths (Shelke et al., 2020). The extracts of *Azadirachta indica* Juss. (Meliceae) had promising anthelmintic properties (F. P. Joshi, 2022). *Warburgia ugandensis* Sprague subsp.ugandensis (Canelaceae) extracts are effective against helminths only that their safety is still unknown (Liu et al., 2020). *Portulaca quadrifida* L. (Portulacaceae) have many pharmacological

benefits including having anthelmintic action (Ahmadou et al., 2022). The used plant parts mentioned as anthelmintics were Bark, stems, seeds, fruits, flowers and roots. However, leaves were the most mentioned parts. The phytochemical compositions vary with season of the year, nature of soils and stage of growth of the plants however phytochemicals tend to be concentrated in barks and leaves compared to other parts (Patle et al., 2020). The preparation methods were crushing or pounding and adding water or soaking in water, this is simulated to using of a polar solvent in extraction. However, *Solanum incunum* was dried, burned to make ashes that were added to drinking water as anthelmintic, this method of preparation and use is different from what is common in literature. Anthelmintic properties of the extracts of Solanaceae are reported not their ashes (Ono et al., 2021).

Chicken owners showed commendable knowledge of use of EVM in managing chicken diseases (average mean knowledge score of 72.5%). This is in agreement with (Ssenku et al. 2022; Schultz et al. 2020; Ebifa-Othieno et al. 2017; Ojelel et al. 2019) who found that people had good ethno-medicine knowledge bases. Most farmers 308 (77%) knew that though plant alternatives have advantages but could lead to adverse effects during use. Majority of the chicken owners were able to propose the causes of adverse effects. Majority expressed knowledge of preparation, propagation and administration. The model couldn't predict chicken owners' knowledge although residence alone could significantly predict the chicken owners' knowledge ($P<0.05$). Although there was no significant residence difference in knowledge chicken owners from rural sub-counties tended to be more knowledgeable compared to those from urban settings possibly because of their easier access to various medicinal plants. The urban chicken owners are more likely to access conventional chicken drugs compared to their rural counterparts. The good scope of knowledge implies the effectiveness of family informal trainings and how EVM is esteemed by communities. This also indicates the interest and the acceptability of EVM. Participatory approaches of identifying alternative chicken medicines are recommended since chicken owners have empirical experiences of the plant alternative medicines.

Chicken owners were convinced about the action of EVM against chicken helminths. Majority of the chicken owners feel that EVM are useful alternatives to synthetic chicken medicines (average mean attitude score of 78%). This is in agreement with (Ebifa-Othieno et al. 2017; Gumisiriza et al. 2021; Abera& Mulate, 2019) who found

the people agreeable with the use of ethno-medicine. The chicken owners were satisfied that EVM are safer than synthetic chicken medicines to chicken and the final consumers. EVM are appropriate alternatives for people that opts for organic chicken farming. Residence alone could predict chicken owners' attitudes ($P<0.01$). Knowledge was a significant predictor of attitudes ($P<0.01$). Chicken owners from rural sub-counties showed better attitudes towards EVM compared to those from urban settings. Education doesn't affect the use of EVM in chicken because it's generally considered effective and acceptable. Chicken owners are willing to promote EVM regardless of the inculcated myths and fallacies. This alternative medicine is hinged on particular traditions which are usually more trusted than synthetic drugs. The observed attitudes as expressed by willingness to preserve, plant, promote and support research in EVM is an indicator that the practice is esteemed.

About half of the poultry farmers in Soroti district population practice EVM (average practice score of 51.2%) and this is in agreement with studies elsewhere (Chaturvedani et al. 2016; Adeleye et al. 2021; Sharma et al. 2022) that found the use of ethno-medicine to be a common practice. Although chicken owners know and are positive about the use of EVM; some of them are unwilling to practice it. The products are not processed, they contain other undesired substances which could have toxic effects. The common adverse effects make the users very careful or even fail to use EVM but would have used if the products were processed and rendered empirically safe. Age alone could predict the practices of chicken owners' ($P<0.05$), older people use EVM compared to young ones. Knowledge alone could predict the practices ($P<0.01$), knowledge determines effective preparations and use. Attitudes could predict the practices ($P<0.01$), those who are convinced by the effectiveness and advantages are the one most likely to use EVM.

The study found that ethanolic extracts of CPL and CAF showed more compounds than their acetone extracts. This relates to the claims of anthelmintic action by chicken owners who soak the crushed plant materials in water which is a polar solvent. A polar (ethanol) and a moderately polar solvent were selected for that reason. Steroids are said to have anthelmintic effects (Wang et al. 2010) but the action all specific lipids on helminths are not fully known. Vitamin C doesn't have particular action against helminths but it improves the response of hosts to helminths infections (Ince et al 2010). Triterpenoids have known effects on helminths (Chama et al. 2020; Kuzminac et al.

2023), however the specific effect of D: A-Friedooleanan-3-ol, (3, alpha.)-and Supraene against helminths requires further investigation. The action of pyranones against helminths are not known and requires further investigation. Phenolics have known action against helminths (Mukherjee et al. 2016; Ndjonka et al. 2014), however the particular effect of Phenol, 5-ethenyl-2methoxy- on helminths is not known. Particular glycosides especially flavonal glycosides have anthelmintic action (Kozan & Anul, 2013) however the action of all other types of glycosides against helminths require further research. Diterpenoids have known action against helminths (Crusco et al. 2018). Phytol is known to be effective against helminths (de Moraes et al. 2014), but the effect of Neophytadiene on helminths requires further research. The action of alkanes against helminths are not known and require further research. Alkaloids have known action against helminths (Dubois et al. 2019; da Silva et al. 2021); Capsaicin and Dihydocapsaicin are known to be effective against helminths especially due to their pungency action (Coronel et al. 2022).

The study found that no deaths were observed nor any observable lesions on the *A. galli* dermis, however inhibited motility was observed with no change of colour of worms. The extracts were significantly effective compared to PBS but acted slowly compared to piperazine citrate during the *in vitro* assays. The action of *Carica papaya* L. extracts is in agreement with the findings of Nghonjuyi et al., (2020) and Sugiharto, (2020). The findings regarding *Capsicum annuum* L. extracts are in agreement with Gentiles et al. (2019) who reported the high anthelmintic potency of *Capsicum annuum* var. Longum. All extracts were effective because they caused above 50% faecal egg count reductions in the *in vivo* studies; however, the *Carica papaya* L. extracts were superior to those of *Capsicum annuum* L. CPLa were distinctly superior to CPLe in faecal egg reductions. The superior action was attributed to the vitamin C in presence of other anthelmintic compounds. The action of vitamin C on helminths in presence of various types of anthelmintics is not well known although Sengupta et al., (2023) mentions that the observed actions arise from vitamin C enhancing the body defence lines. The findings about the *in-vivo* efficacy of *Carica papaya* L. extracts are in agreement with Sen et al., (2020) . The findings regarding the *in-vivo* action of *Capsicum annuum* L. extracts are in agreement with Gentiles et al., (2019) who observed significant faecal egg reductions.

The study found that compared to PBS; CAFa, CAFe, CPLa and CPLe didn't significantly affect renal function parameters, however, CAFa needs to be used with caution because it was associated with high Na^+ in blood. The extracts didn't show significant effects on the electrolyte balance with regard to BUN, Na^+, Cl^- and creatinine, they are probably safe for use at the given concentrations. Although the extracts didn't affect the Na^+ electrolyte balance, CAFa caused a lower blood Na^+ compared to other extracts and piperazine. Lower blood Na^+ predisposes to decreased blood pressure, increased uric acid, increased haematocrit, shock and heart failure (Julian, 1987; Sad, 2016). Use of CAFa requires concomitant use of diuretics or with timely monitoring of electrolyte balance for action. The plant extracts were as safe as the synthetic anthelmintics regarding effects on the BUN, Na^+, Cl^- and creatinine balance. The extracts, piperazine citrate and levamisole hydrochloride had no effects on the metabolic actions that yields uric acid. This is in agreement with the finding that chickens have mechanisms to maintain serum uric acid balance which is related to glucose transporter 9 (GLUT9) although the underlying pathways are not yet well understood (Ding et al., 2021).

The placebo with higher helminths loads had less blood eosinophils compared to the treatments possibly because the inflammatory action of the treatments were greater than action of helminths that leads to eosinophilia. Eosinophils are involved in the starting inflammatory responses, they can act as antigen presenting cells, are particularly responsible for defence against parasite infections and they modulate host's general immune responses (Rothenberg & Hogan, 2006). Like piperazine and levamisole; the extracts possibly induced hypersensitivity, autoimmune disorders or neoplasms defence mechanisms which manifest as eosinophilia (Kanuru & Sapra, 2023), and however the pathways require further investigation. However further study on how ethnoveterinary anthelmintics affect the immune system should be done. The current study deduced that the studied ethnoveterinary anthelmintics were safe to use at the stated dosage. The plant extracts were as safe as the synthetic anthelmentics regarding effects on the haematological parameters balance.

The different extracts didn't show any observable lesions on the heart of chickens. The anthelmintic extracts are not toxic to the heart muscles and major vessels. The non-cardiac toxicity showed by *Capsicum annuum* L. (acetone red pepper extract & ethanol

red pepper extract) is in agreement with Mandal et al., (2023) who reported that *Capsicum annuum* L. is cardio-protective. Sanati et al. (2017) also reported that *Capsicum annuum* L. has beneficial effects on the heart. The findings about the action of *Carica papaya* L. (acetone pawpaw leaves extract & ethanol pawpaw leaves extract) are in agreement with Haramaki et al., (1995); Hasimun et al., (2020); Kong et al., (2021) who reported about the cardio-protective action of *Carica papaya* L. Just as the experimented extracts, piperazine and levamisole didn't cause any observable lesions on the chicken hearts. Acetone red pepper extract (CAFa), ethanol red pepper extract (CAFe), and acetone pawpaw leaves extract (CPLa) were toxic to kidneys. The actions of *Capsicum annuum* L. are in agreement with Jabar & Jassim, (2023) who observed kidney histological alterations among animals treated with *Capsicum annuum* L.. Yuca, (2022) also reported kidney damages among animals that received very high doses of *Capsicum annuum* L. The reasons why CPLa was toxic are not known because like CPLe, *Carica papaya* L. is reported in the literature as nephro-protective (Francis et al. 2020). It's speculated that acetone which is a moderately polar solvent yields some compounds with nepro-toxic effects unlike ethanol which is highly polar. However further investigations are needed to prove the variations in the compounds yielded with the different solvents and the renal effects of such compounds. Levamisole was equally toxic to the kidneys but piperazine was safe. Nephro-toxic effects of levamisole have been reported in mice (Almawla & Al Baggou, 2023), however there are no such earlier reports in chicken. There is need to confirm the findings and guide regarding the safety of levamisole in chicken at the efficacious concentration. All extracts were toxic to the liver except CPLe, equally piperazine and levamisole were toxic to chicken liver. The reports regarding *Capsicum annuum* L. are in disagreement with Das et al., (2018); Effendi & Sukmanadi, **(**2021); Oloruntola et al., (2024) who reported the hepato-protective actions of *Capsicum annuum* L in other species. The concentrations in this experiment were very high compared to the concentrations in literature where *Capsicum annuum* L. is reported to have had protective action. *Carica papaya* L. is also reported to be hepato-protective as in CPLe (Awodele et al. 2016; Shaban et al. 2021). However, CPLa on the contrary was toxic because of the difference in compound yields of acetone compared to ethanol. The findings about the effects of piperazine were in agreement with those of Bakhrebah et al. (2011) who observed histological changes in the chicken liver. The findings regarding levamisole were in agreement with Miah et al. 2020 who reports that high doses of levamisole are detrimental. Generally, all

extracts were toxic except CPLe, levamisole was also toxic and piperazine was moderately toxic. CPLe was as safe as the placebo, safer than the two synthetic anthelmintics (piperazine and levamisole) that are commonly used by commercial chicken farmers in Uganda.

8.1 Conclusions

The participant knowledge on EVM in chicken is insufficient and predisposes the chicken to possible adverse events. Although EVM are acceptable and popular the users are concerned about adverse effects. The practices are woven on the coherence theory and need to be tested based on the Bayesian theorem which gives a critical holistic evaluation. It's likely that some plants have anthelmintic properties apparently based on the consensus theory. Chicken owners demonstrated a good knowledge base and were able to use EVM as alternative chicken medicine. The chicken owners were convinced about the effectiveness of EVM. EVM were acceptable by the various chicken owners' gender, age groups and educational levels. Even with the good average knowledge and attitude score, the average practice score was low. The findings were in tandem with the stated theories of the study of ethnomedicines.

Capsicum annuum fruits contained high concentrations sterols and alkaloids while *Carica papaya* leaves contained sterols, terpenoids and phenolics. Pyranones were in relatively high concentrations in both CAF and CPL although their anthelmintic action is unknown. The CPLa was faster than CPLe. It was only CPLa that contained vitamin C. All Carica papaya L. extracts had terpenoids whose action against nematodes is said to be boosted by vitamin C. CPLa was as effective as levamisole, was different from CPLe, CPLa had higher concentration of vitamin C. The extracts inhibited motility of *Ascaridia galli* but acted slowly compared to piperazine citrate. All extracts had higher FECR than piperazine citrate and levamisole hydrochloride caused the highest faecal egg count reduction. The results show that there is no levamisole hydrochloride anthelmintic resistance in chicken in the study area although piperazine citrate anthelmintic resistance is likely. I rejected the null hypothesis because the extracts were effective.

Organ toxicity study showed that CPLe was the safest extract. Other extracts caused observable organ toxicity lesions in the order of CAFa < CPLa < CAFe. The study empirically points the relative toxicity of piprazine citrate and levamisole hydrochloride

at recommended concentrations compared to plant extracts used as anthelmintics in chicken. The extracts were toxic to kidneys and liver. There was no observed difference in safety between the plant anthelmentics and the synthetic anthelmentics (piperazine citrate & levamisole hydrochloride), all should be used with caution. I couldn't reject the null hypothesis because the extracts were not safe.

8.2 General recommendations

1. Medicinal plants planting and gazetting major sources, Participatory approaches of identifying alternative chicken medicines.

2. Action of pyranones on helminths should be experimented, investigating the role vitamin C in herbal anthelmintic actions.

3. Testing all plant candidates against AH resistant worms, efficacy studies in different breeds of chicken.

4. AH should be used with caution and meticulous monitoring

8.3 Suggested future Research

1. Efficacy and safety experiments on all the mentioned plant candidates
2. Use of herbal-synthetic antihelmintic combinations
3. Efficacy experiments on piperazine-vitamin C combinations in chicken.
4. KAP studies on treating the different specific chicken diseases using ethnoveterinary medicine.
5. Efficacy and safety studies of highly purified *Carica papaya* L. and *Capsicum annuum* L. extracts.
6. Fractionating and structural elucidation of the effective fractions of CPLa.

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APPENDICES

Appendix I: Guiding questions for the farmers Focus Group Discussions

Tool Number: FGD-01-GZ

Dear ladies and gentlemen. We are a research team from Makerere University, school of Veterinary and Animal resources, college of veterinary medicine, Animal resources and biosecurity, Kampala. We are requesting to discuss with you how you manage diseases of chicken especially using non allopathic medicines. We shall use identifiers and avoid use of names to maintain the confidentiality of the information. The information is for study purposes. The exercise is based on an understanding and participation is absolutely voluntary.

a) **Respondents data**

1. Parish…….
2. Sub-county…….
3. District…….
4. Coordinate……..
5. Number of males……Number of females……
6. Average age of participants……

b) **Use of ethnoveterinary practices**

1. Do you use or know anyone that uses EVP in controlling chicken diseases?
2. What plants, parts, local names, diseases are they effective against?-Listing following the order of importance of the diseases, starting with the most important.
3. What plants are effective against chicken helminths?
4. Do the EVP lead to any adverse effects?
5. What are the reasons for the adverse effects?
6. Give participants time to walk us through a transect before sitting to agree on availability, challenges in access/use and how the information is acquired/disseminated.

Thank you for the time

Appendix II: Guiding questions for the farmer questionnaires

Tool Number: FI-01-GZ

a) Respondent data

1) Name of respondent……..
2) Sex of respondent…….....
3) Level of education: *a) Pre-primary b) Primary c) O-level d) A-level e) Certificate f) Diploma g) Bachelors h) Masters*
4) Parish……...
5) Sub-county……..
6) Coordinate……...
7) Average monthly income from chicken in UGX: ……..
8) Average monthly expenditure on chicken in UGX:……

b) Poultry Inventory

1) Species of birds reared and numbers:…….....
2) How often do you deworm your birds?.....
3) How do you know that chicken have worms?.....
4) Which dewormers do you use?.....

c) Use of ethnoveterinary materials

1) Do you use ethnoveterinary materials? *a) Yes b) No*
2) Do you know other farmers that use ethnoveterinary materials? *a) Yes b) No*
3) If yes, what materials are used……
4) Mention the plant, local name, plant part and methods of preparation and application.

Plant	*Local name*	*Plant part*	*Disease targeted*	*Methods of preparation*	*Methods of application*

5) Are ethnoveterinary materials effective against chicken helminths? Why?.....
6) How did you come up with the regimen for ethnoveterinary materials?.....
7) How do you acquire all the ethnoveterinary materials listed?.....

8) Have you ever noticed any adverse effects in the birds after drug administration? *a) Yes b) No*

9) Explain the adverse effects:……

10) What was possibly the reason for the adverse effects observed?.....

11) How did you acquire this knowledge?.....

12) Do you freely share this information with everyone? *a) Yes b) No*

13)

d) Other Chicken treatment practices

e)

1) Do you use human drugs to treat chicken? *a) Yes b) No*
2) If yes, which drugs and against what diseases or parasites

Drug	*Constitution and quantity*	*Disease/parasite*

Thank you for your time

Appendix III: Knowledge, Attitudes and Practices Items

	Item for Knowledge (Table 3a)	True	False	I don't know
$K_{1.}$	Ethnoveterinary medicine in chicken is the use of plant materials to treat different diseases.	376 (92.4%)	9 (2.2%)	22 (5.4%)
$K_{2.}$	Ethnoveterinary medicine in chicken involves picking the specific plant materials and processing them in a specific way	370 (91%)	11 (2.7%)	26 (6.3%)
$K_{3.}$	I am cognizant of the fact that different ages and sizes of chicken should be given different volumes of the ethnoveterinary medicines.	363 (89.2%)	16 (3.9%)	28 (6.9%)
$K_{4.}$	I am aware that though Ethnoveterinary materials are good yet could result in undesired adverse effects.	313 (77%)	41 (10%)	53 (13%)
$K_{5.}$	The possible adverse effects after administration of ethnoveterinary medicines in chicken may be because of wrong preparation methods.	302 (74.2%)	45 (11.1%)	60 (14.7%)
$K_{6.}$	The possible adverse effects after administration of ethnoveterinary medicines in chicken may be because of giving the drug for a long period (3-7) days.	247 (60.7%)	86 (21.1%)	74 (18.2%)
$K_{7.}$	The possible adverse effects after administration of ethnoveterinary medicines in chicken may be because of giving the drug to very sick birds.	253 (62.2%)	84 (20.6%)	70 (17.2%)
$K_{8.}$	The possible adverse effects after administration of ethnoveterinary medicines in chicken may be because of giving very high concentrations to the birds.	286 (70.3%)	42 (10.3%)	79 (19.4%)
$K_{9.}$	The African custard apple (ebwolo) bark concoction is effective against Newcastle disease.	197 (48.4%)	25 (6.1%)	185 (45.5%)
$K_{10.}$	The Ugandan green heart (abacci) is used to make a solution effective against diarrheoa	254 (62.6%)	23 (5.7%)	129 (31.7%)
$K_{11.}$	The local soda ash (emagadi) made from aboga and soddom ash is effective against ectoparasites	246 (60.4%)	23 (5.7%)	138 (33.9%)
$K_{12.}$	I can prepare the ethnoveterinary material for the treatment of weak chicken	305 (75.3%)	31 (7.7%)	69 (17%)
$K_{13.}$	I can propagate the plants which are the main sources of the ethnovetrinary materials used in poultry	316 (77.6%)	36 (8.8%)	55 (13.5%)

K_{14}.	I know how to administer the ethnoveterinary medicines to my chicken	328 (80.8%)	25 (6.1%)	53 (13.1%)
K_{15}.	Response to adverse events due to administration of ethnoveterinary materials requires immediate withdrawing and administration of plain water.	264 (64.9%)	46 (11.3%)	97 (23.8%)
K_{16}.	I know the appropriate sources of the ethnoveterinary materials that I use on my chickens.	315 (77.6%)	24 (5.9%)	67 (16.5%)
	Item for Attitudes (Table 3b)	**Agree**	**Disagree**	**I don't know**
A_1.	Do you agree that ethnoveterinary medicines are effective against chicken diseases	346 (85.6%)	19 (4.7%)	39 (9.7%)
A_2.	Do you agree that use of ethnoveterinary medicines in chicken is associated with myths and fallacies	250 (61.4%)	67 (16.5%)	90 (22.1%)
A_3.	Do you agree with the promotion of ethnoveterinary medicines in chicken by the government?	353 (87%)	35 (8.6%)	18 (4.4%)
A_4.	Do you agree that research in the use of ethnoveterinary medicines in chicken is useful	379 (93.1%)	11 (2.7%)	17 (4.2%)
A_5.	Do you agree that ethnoveterinary medicines are alternatives to the conventional chicken medicines	349 (86%)	27 (6.6%)	30 (7.4%)
A_6.	Do you agree that national policies that protect natural forests are important to promote ethnoveterinary medicines in chicken	367 (90.2%)	11 (2.7%)	29 (7.1%)
A_7.	Do you agree with the planting of ethnoveterinary plant materials on compounds and in the gardens of chicken owners?	376 (92.4%)	13 (3.2%)	18 (4.4%)
A_8.	Do you agree that chicken raised on ethnoveterinary drugs are safer to consumers than those raised conventional synthetic drugs?	313 (76.9%)	50 (12.3%)	44 (10.8%)
A_9.	Do you agree that chicken on organic farms can depend solely on ethnoveterinary medicines and be successful?	268 (66%)	89 (21.9%)	49 (12.1%)
A_{10}.	Do you agree that what chicken herbalists sell are genuine chicken ethnoveterinary medicines?	180 (44.4%)	136 (33.6%)	89 (22%)
	Item for Practices (Table 3c)	**Always**	**Sometimes**	**Never**
P_1.	I use ethnoveterinary medicines on my chickens	115 (28.3%)	265 (65.1%)	27 (6.6%)

P_2.	I plant my ethnoveterinary medicine plant sources	111 (27.3%)	244 (60%)	52 (12.7%)
P_3.	I share my ethnoveterinary medicine knowledge	141 (34.6%)	236 (58%)	30 (7.4%)
P_4.	I study the effects of the administered ethnoveterinary medicines in my chicken	160 (39.3%)	197 (48.4%)	50 (12.3%)
P_5.	I determine doses of my ethnoveterinary medicines that I administer to chicken	210 (51.7%)	149 (36.7%)	47 (11.6%)
P_6.	I attend farmer trainings to learn ethnoveterinary medicines of chicken	86 (21.2%)	212 (52.2%)	108 (26.6%)
P_7.	I buy my ethnoveterinary for my chicken from the markets	62 (15.3%)	232 (57.1%)	112 (27.6%)
P_8.	I interact with village elders to learn ethnoveterinary medicines of chicken	131 (32.2%)	220 (54%)	56 (13.8%)
P_9.	I record my ethnoveterinary materials and the methods of application in chicken	68 (16.7%)	153 (37.6%)	186 (45.7%)
P_{10}.	I wash my hands when preparing ethnoveterinary medicines and I wash the plants before processing	240 (59%)	140 (34.4%)	27 (6.6%)
P_{11}.	I soak my ethnoveterinary materials in cold water	256 (62.9%)	116 (28.5%)	35 (8.6%)
P_{12}.	I boil my ethnoveterinary materials	75 (18.4%)	205 (50.4%)	127 (31.2%)
P_{13}.	I give to my chicken the same preparation of ethnoveterinary medicines for several days (3-5) days.	115 (28.3%)	173 (42.5%)	119 (29.2%)
P_{14}.	I use human drugs alongside the ethnoveterinary medicines	43 (10.6%)	168 (41.3%)	196 (48.1%)
P_{15}.	I add alcohol or energy drinks or honey to the ethnoveterinary medicines	28 (6.9%)	122 (30%)	257 (63.1%)
P_{16}.	I follow particular rituals when administering ethnoveterinary medicines to chicken	39 (9.6%)	101 (24.8%)	267(65.6%)

Appendix IV: Predictors of chicken owners' Knowledge of EVM for the control of chicken diseases

Predictor	***df***	***SE***	***t* value**	***B***	***b***	***P* value**
Gender	4	.400	1.234	*.493*	.062	.218
Residence	4	.128	-2.121	-.271	-.108	.035
Age	4	.341	.298	.101	.015	.766
Education	4	.226	-1.511	-.341	-.080	.132
R^2*=.018*						
rR^2*=.008*						
F=.126						

N = 408; *df* = level of freedom; *SE* = Standard error; *B* = Regression coefficient; *b* = Standard β.

Appendix V: Predictors of chicken owners' Attitudes of EVM for the control of chicken diseases

Regression Model	Predictor	*df*	*SE*	*t* value	*B*	*b*	*P* value
Model 1	Gender	4	0.22	0.013	*0.003*	0.001	0.99
	Residence	4	0.07	-2.999	-0.21	-0.15	0.003
	Age	4	0.19	0.678	0.128	0.035	0.498
	Education	4	0.13	0.764	0.096	0.04	0.445
	R^2*=.026*						
	rR^2*=.016*						
	F=.031						
Model 2	Gender	5	0.2	-0.603	-0.12	-0.03	0.547
	Residence	5	0.06	-2.275	-0.15	-0.1	0.023
	Age	5	0.17	0.608	0.103	0.028	0.544
	Education	5	0.11	1.605	0.18	0.076	0.109
	Knowledge	5	0.03	10.037	0.248	0.446	0
	R^2*=.222*						
	rR^2*=.212*						
	F=.000						

N= 408; *df* = level of freedom; *SE* = Standard error; *B* = Regression coefficient; *b* = Standard β; **p* < **.05**; ***p* < **.01**.

Appendix VI: Predictors of chicken owners' Practices of EVM for the control of chicken diseases

Regression Model	Predictor	*df*	*SE*	*t* value	*B*	*b*	*P* value
Model 1	Gender	5	0.61	-0.94	*-0.57*	-0.05	0.348
	Residence	5	0.19	-1.752	-0.34	-0.09	0.081
	Age	5	0.52	-2.493	-1.29	-0.12	0.013
	Education	5	0.34	1.531	0.525	0.077	0.127
	Knowledge	5	0.08	5.653	0.426	0.268	0
	R^2=.111						
	rR^2=.100						
	F=.000						
Model 2	Gender	6	0.6	-0.842	-0.5	-0.04	0.401
	Residence	6	0.19	-1.343	-0.26	-0.07	0.18
	Age	6	0.51	-2.647	-1.34	-0.13	0.008
	Education	6	0.34	1.249	0.423	0.062	0.212
	Knowledge	6	0.08	3.445	0.286	0.18	0.001
	Attitudes	6	0.15	3.775	0.566	0.198	0
	R^2=.142						
	rR^2=.129						
	F=.000						

N= 408; *df* = level of freedom; *SE* = Standard error; *B* = Regression coefficient; *b* = Standard β; **p* < **.05**; ***p* < **.01**.

Appendix VII: Multiple comparisons of *In-vivo* Efficacy tests of the extracts, Piperazine and Levamisole

Dependent Variable: FECR

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
PBS	CPLa	-97.67*	1.533	.000	-102.90	-92.43
	CAFa	-79.67*	1.533	.000	-84.90	-74.43
	CPLe	-54.00*	1.533	.000	-59.23	-48.77
	CAFe	-76.33*	1.533	.000	-81.57	-71.10
	Lev	-98.67*	1.533	.000	-103.90	-93.43
	Pip	-35.67*	1.533	.000	-40.90	-30.43
Pip	CPLa	-62.00*	1.533	.000	-67.23	-56.77
	CAFa	-44.00*	1.533	.000	-49.23	-38.77
	CPLe	-18.33*	1.533	.000	-23.57	-13.10
	CAFe	-40.67*	1.533	.000	-45.90	-35.43
	Lev	-63.00*	1.533	.000	-68.23	-57.77
	PBS	35.67*	1.533	.000	30.43	40.90
Lev	CPLa	1.00	1.533	.993	-4.23	6.23
	CAFa	19.00*	1.533	.000	13.77	24.23
	CPLe	44.67*	1.533	.000	39.43	49.90
	CAFe	22.33*	1.533	.000	17.10	27.57
	PBS	98.67*	1.533	.000	93.43	103.90
	Pip	63.00*	1.533	.000	57.77	68.23

All extracts were significantly more effective compared to PBS (p =0.000), all extracts caused higher FECR than piperazine citrate *(p=0.000)* and CPLa was as good as levamisole *(p=0.993)*

Appendix VIII: Multiple Comparisons of Renal functions

(I)Treatment	(J) Treatment	Mean±SD	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Dependent Variable: BUN					
Control	CPLa	3.413±5.61	0.994	-8.6264	12.5397
	CAFa	7.118±4.36	0.997	-12.3297	8.8364
	CPLe	5.767±3.23	1	-10.9797	10.1864
	CAFe	10.567±2.16	0.64	-15.7797	5.3864
	Leva	5.710±3.83	1	-10.923	10.243
	Pip	5.197±2.07	1	-10.4097	10.7564
	Control	5.370±4.05			
Dependent Variable: Sodium					
CAFa	CPLa	24.213±3.83	0.058	-17.9335	0.2335
	Control	23.440±5.51	0.097	-17.1601	1.0068
	CPLe	24.583±4.20	.046*	-18.3035	-0.1365
	CAFe	27.967±2.38	.005**	-21.6868	-3.5199
	Leva	24.343±2.02	0.054	-18.0635	0.1035
	Pip	24.773±1.25	.040*	-18.4935	-0.3265
	CAFa	15.363±0.55			
Control	CPLa	24.213±3.83	1	-9.8568	8.3101
	CAFa	15.363±0.55	0.097	-1.0068	17.1601
	CPLe	24.583±4.20	0.999	-10.2268	7.9401
	CAFe	27.967±2.38	0.626	-13.6101	4.5568
	Leva	24.343±2.02	1	-9.9868	8.1801
	Pip	24.773±1.25	0.998	-10.4168	7.7501
Dependent Variable: Chloride					
Control	CPLa	92.667±2.87	0.976	-5.035	8.301
	CAFa	97.733±2.25	0.593	-10.101	3.235

	CPLe	95.167±4.10	0.999	-7.535	5.801
	CAFe	94.433±1.93	1	-6.801	6.535
	Leva	96.833±1,51	0.842	-9.201	4.135
	Pip	94.533±1.08	1	-6.901	6.435
	Control	94.300±1.65			
Dependent Variable: Creatinine					
Control	CPLa	1.177±0.35	0.496	-0.9425	0.2625
	CAFa	0.860±0.11	1	-0.6258	0.5791
	CPLe	0.700±0.17	0.984	-0.4658	0.7391
	CAFe	1.250±0.16	0.298	-1.0125	0.1925
	Leva	0.843±0.13	1	-0.6091	0.5958
	Pip	0.853±0.28	1	-0.6191	0.5858
	Control	0.837±0.22			
Dependent Variable: Uric acid					
Control	CAFa	0.100±0.05	0.988	-0.13996	0.21596
	CAFe	0.178±0.04	0.984	-0.21829	0.13762
	CPLa	0.106±0.04	0.995	-0.14629	0.20962
	CPLe	0.127±0.03	1	-0.16696	0.18896
	Lev	0.112±0.06	0.998	-0.15196	0.20396
	Pip	0.161±0.13	0.999	-0.20062	0.15529

There was no significant difference in (BUN, sodium, chloride, Creatinine and uric acid) when comparing the treatments and control birds. However, CPLe caused significantly higher blood sodium than CAFa (p=0.046), CAFe caused higher blood sodium than CAFa (p=0.005), piperazine citrate caused higher blood sodium than CAFa (p=0.04)

Appendix IX: Multiple Comparisons of Liver Function Parameters

(I) Group	(J) Group	Mean ± SD	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Dependent Variable: Albumin					
CAFe	CAFa	13.633±0.72	0.56	-5.268	1.602
	CPLa	13.233±1.38	0.78	-4.868	2.002
	CPLe	15.733±0.71	.020*	-7.368	-0.498
	Lev	12.633±0.51	0.98	-4.268	2.602
	PBS	15.100±1.71	0.06	-6.735	0.135
	Pip	14.733±1.30	0.12	-6.368	0.502
	CAFe	11.800±1.68			
Dependent Variable: AST					
Lev	CAFa	263.167±21.4	0.07	-6.001	194.001
	CAFe	253.667±46.26	.040*	3.499	203.501
	CPLa	276.333±49.64	0.15	-19.168	180.835
	CPLe	324.867±49.79	0.92	-67.701	132.301
	PBS	261.267±36.60	0.06	-4.101	195.901
	Pip	268.567±8.81	0.1	-11.401	188.601
	Lev	357.167±6.88			

CPLe caused a significantly higher blood albumin compared to CAFe (p=0.02), Levamisole hydrochloride caused a significantly higher blood AST compared to CAFe (p=0.004).

Appendix X: Multiple Comparisons of Haematological parameters in chickens treated with herbal and synthetic anthelmintics

(I) Group	(J) Group	Mean±SD	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Dependent Variable: PCV %					
Control	CPLa	25.667±4.04	1	-9.448	9.115
	CAFa	26.167±2.93	1	-9.948	8.615
	CPLe	28.667±4.93	0.896	-12.448	6.115
	CAFe	30.500±4.09	0.545	-14.282	4.282
	Lev	25.500±2.50	0.788	-13.115	5.448
	Pip	30.000±1.00	0.653	-13.782	4.782
	Control	29.333±2.08			
Dependent Variable: TPP g/dL					
Control	CPLa	3.167±0.21	0.404	-0.4925	2.0591
	CAFa	3.200±0.36	0.451	-0.5258	2.0258
	CPLe	3.033±1.01	0.247	-0.3591	2.1925
	CAFe	4.133±0.11	0.999	-1.4591	1.0925
	Lev	3.950±0.35	0.604	-0.6258	1.9258
	Pip	3.567±0.25	0.939	-0.8925	1.6591
	Control	3.300±0.26			
Dependent Variable: Fib g/dL					
Control	CPLa	0,100±0.00	0.924	-0.1081	0.2081
	CAFa	0.233±0.06	0.568	-0.2414	0.0747
	CPLe	0.100±0.00	0.924	-0.1081	0.2081
	CAFe	0.133±0.06	1	-0.1414	0.1747
	Lev	0.150±0.05	1	-0.1414	0.1747
	Pip	0.200±0.10	0.924	-0.2081	0.1081
	Control	0.133±0.06			

Dependent Variable: TWBC/uL					
Control	CPLa	5.733±2.87	1	14.6439	13.2772
	CAFa	9.067±1.32	0.95	17.9772	9.9439
	CPLe	10.10±11.02	0.869	19.0105	8.9105
	CAFe	10.633±4.41	0.81	19.5439	8.3772
	Lev	5.050±1.95	0.994	16.6105	11.3105
	Pip	7.233±1.94	0.998	16.1439	11.7772
	Control	7.700±4.12			
Dependent Variable: TRBC/uL					
Control	CPLa	2.833±0.25	1	-2.5958	3.2292
	CAFa	3.267±0.98	1	-3.0292	2.7958
	CPLe	4.000±1.83	0.947	-3.7625	2.0625
	CAFe	4.033±0.51	0.937	-3.7958	2.0292
	Lev	3.150±0.85	0.996	-3.4292	2.3958
	Pip	4.133±0.91	0.9	-3.8958	1.9292
	Control	3.667±1.20			
Dependent Variable: HB g/dL					
Control	CPLa	6.333±1.55	1	-2.8	3.333
	CAFa	7.233±0.38	0.99	-3.7	2.433
	CPLe	7.933±2.01	0.749	-4.4	1.733
	CAFe	6.667±1.17	1	-3.333	2.8
	Lev	6.600±0.60	0.994	-3.633	2.5
	Pip	8.00±0.40	0.708	-4.466	1.666
	Control	7.167±1.04			
Dependent Variable: Eos %					
Control	CPLa	5.000±1.00	.017*	0.74	9.26
	CAFa	2.000±1.00	.000**	3.74	12.26
	CPLe	5.000±2.65	.017*	0.74	9.26
	CAFe	3.000±1.00	.001**	2.74	11.26
	Lev	10.000±2.00	.001**	3.07	11.59
	Pip	2.000±1.00	.000**	3.74	12.26

	Control	2.670±1.16			
Dependent variable: Mon %					
Control	CPLa	8.00±3.61	0.804	-12.75	5.42
	CAFa	4.67±3.06	1	-9.42	8.75
	CPLe	7.67±1.53	0.862	-12.42	5.75
	CAFe	3.67±1.53	1	-8.42	9.75
	Lev	16.00±6.00	.009**	-20.75	-2.58
	Pip	10.00±2.65	0.388	-14.75	3.42
	Control	4.33±2.08			
Dependent Variable: LMP %					
Control	CPLa	61.67±18.56	0.173	-60.6	7.26
	CAFa	39.67±13.80	0.999	-38.6	29.26
	CPLe	48.33±17.50	0.822	-47.26	20.6
	CAFe	39.330±9.24	0.999	-38.26	29.6
	Lev	35.000±1.00	0.893	-45.6	22.26
	Pip	38.00±5.29	1	-36.93	30.93
	Control	46.67±9.02			
Dependent Variable: HTR %					
Control	CPLa	27.33±17.01	0.995	-30.51	43.85
	CAFa	53.00±15.72	0.601	-56.18	18.18
	CPLe	44.67±23.01	0.951	-47.85	26.51
	CAFe	51.000±8.54	0.707	-54.18	20.18
	Lev	34.000±4.00	0.757	-53.18	21.18
	Pip	50.330±2.52	0.741	-53.51	20.85
	Control	50.00±9.17			
Dependent Variable: Band %					
Control	CPLa	4.330±1.53	1	-5.11	6.44
	CAFa	4.000±1.00	0.996	-4.77	6.77
	CPLe	2.000±1.00	0.583	-2.77	8.77
	CAFe	5.670±4.51	1	-6.44	5.11
	Lev	5.000±1.00	1	-5.77	5.77

Pip	2.33±0.50	0.697	-3.11		8.44
Control	5.00±2.00				

CAFa group showed significantly lower eosinophil numbers than the control group (p=0.000). CAFe group showed a significantly more eosinophils than control group *(p=0.01).* CPLa group showed a significantly more eosinophils than control group *(p=0.017).* Piperazine group showed significantly lower eosinophil numbers than the control group (*p=0.000*). Levamisole group showed a significantly more eosinophils than control group *(p=0.001).*

Levamisole group showed a significantly more monocytes than control group *(p=0.009).*

Appendix XI: Most cited plants against chicken helminths

***Carica papaya* L.**

***Capsicum annuum* L.**

Appendix XII: Policy Brief Draft

Guidelines on the use of anthelmintic ethnoveterinary medicine in chicken

Introduction: Ethnoveterinary medicine is getting more popular with the increasing antimrobial resistance and lack Synthetic drugs. However, use of commercial concoctions and ethnoveterinary medicines developed at homes requires extra caution. Though the ethnoveterinary anthelmintics are effective but are not safe. Prolonged use will be detrimental to chicken health, longevity and productivity.

Problem: At just the therapeutic concentration, damages were observed on the major organs of the experimental chickens. The cumulative toxic effects are expected to be worse among chicken farmers who use herbal anthelmintic interventions throughout the chicken production cycles.

Key issues identified

1. Ethnoveterinary anthelmintics are effective at a certain concentration by inhibiting motility. They don't cause mortality of the helminths and have no effects on the dermis of worms.
2. EVM anthelmintics had no effects on the chicken hearts but were toxic to the liver and kidney
3. Not recommended for use in chicks, not for weak chicken and not for continuous use but to be discontinued after three days of use.

Who needs to know?

1. Ministry of Agriculture, Animal Industry and Fisheries (MAAIF).
2. Ministry of Science and Technology
3. Uganda National Council of Higher Education (UNCHE)
4. Uganda Farmers Association (UFA)
5. All education development partners
6. National Planning departments and agencies
7. Uganda National Council of Science and Technology (UNCST)
8. The National Curriculum Development Centre

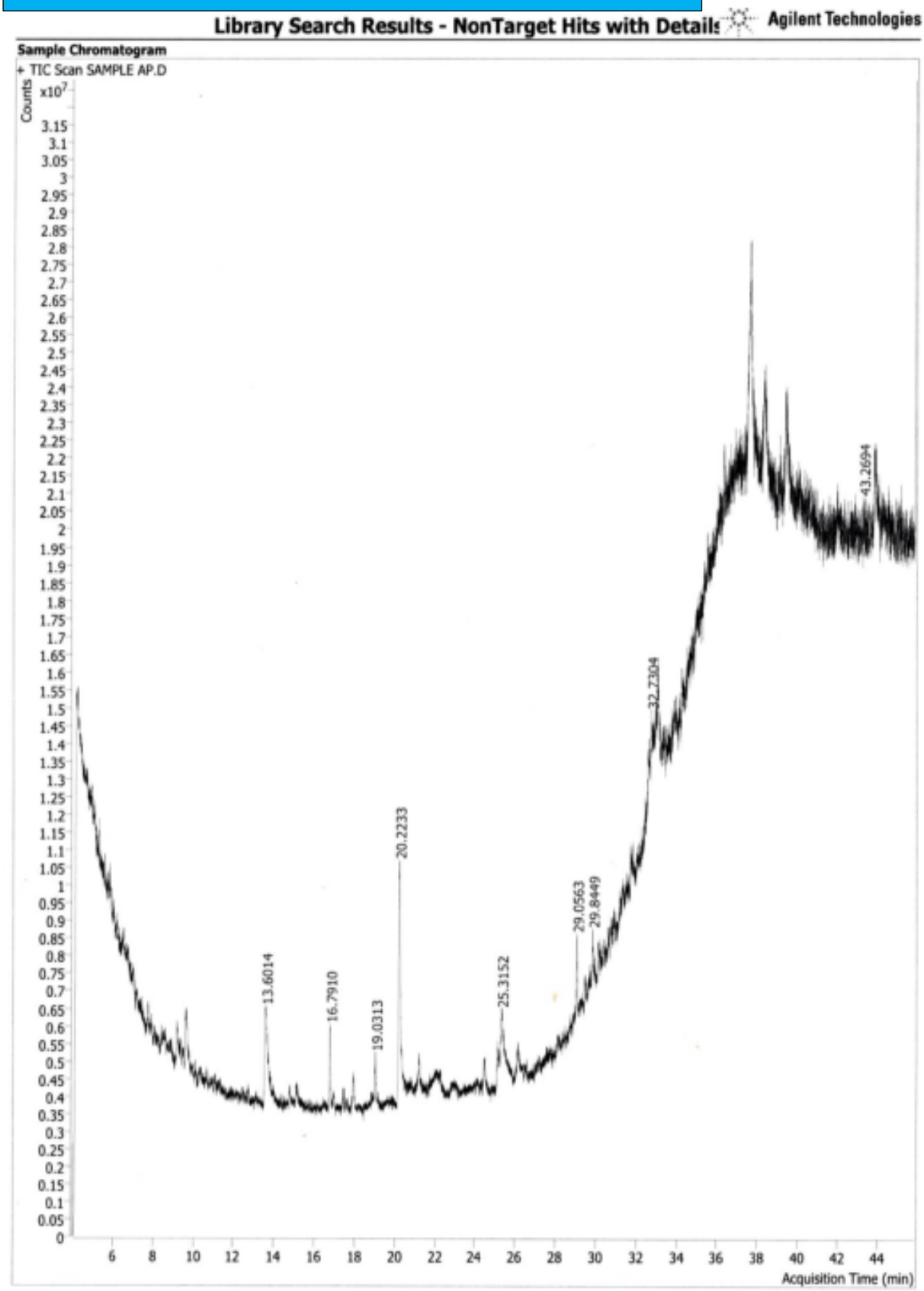
Policy proposals

1. Ethnoveterinary material producers should state the details of doses, toxicity reports, season of harvest and geographical zones of plants used.
2. Ethnovetrinary material processors and packers should state clearly that no synthetic compounds were added.
3. The communities should be engaged to propagate the endangered plant species.
4. There is need of more financing for clinical trials and elucidations.

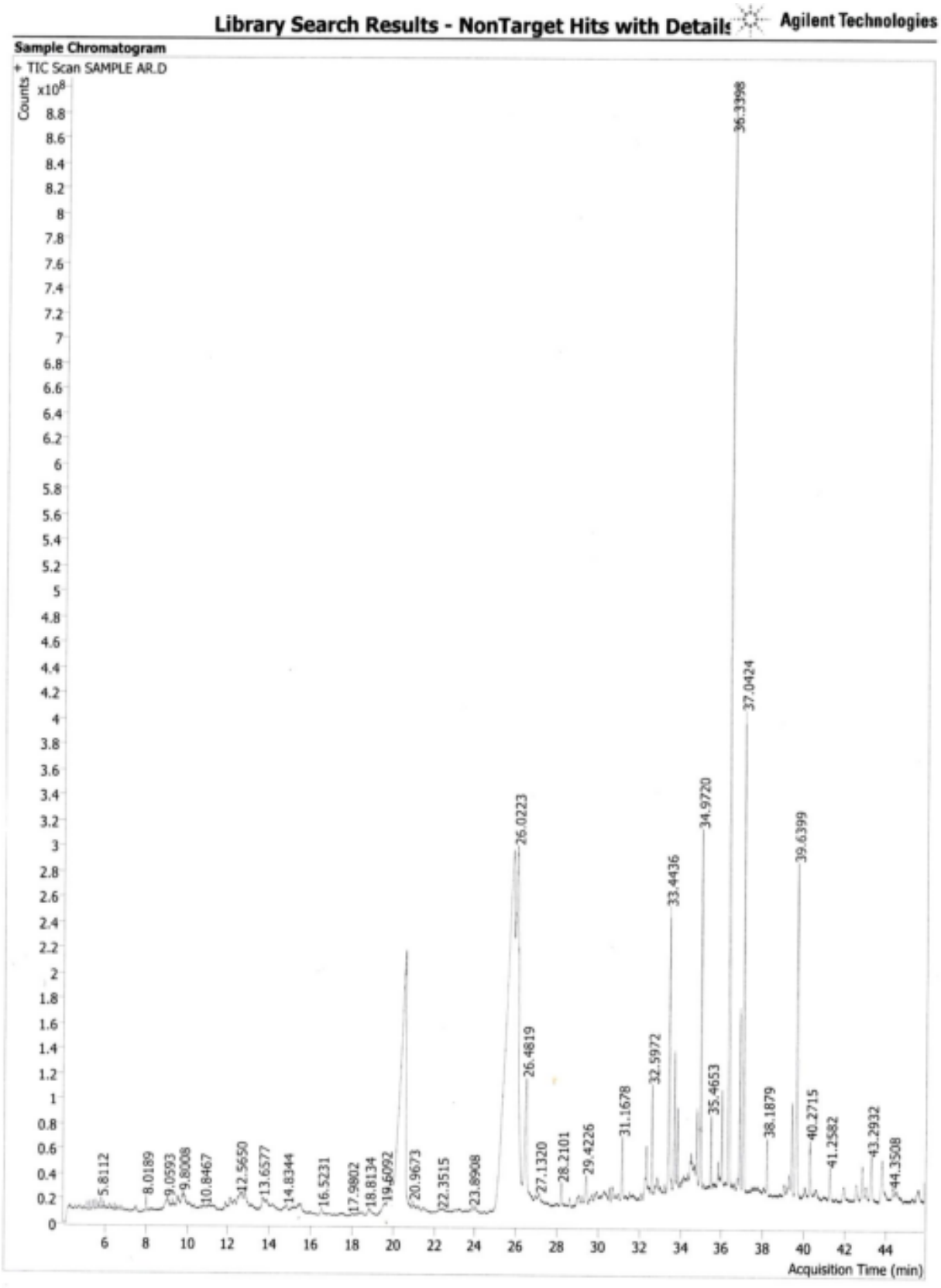
Conclusion

Implementing the policy proposal will protect chicken from toxicity and farmers from being cheated by herbalists and ethnovetrinary medicine dealers. Implementation of the policy will also enhance Uganda's capacity to legislate ethnoveterinary medicine.

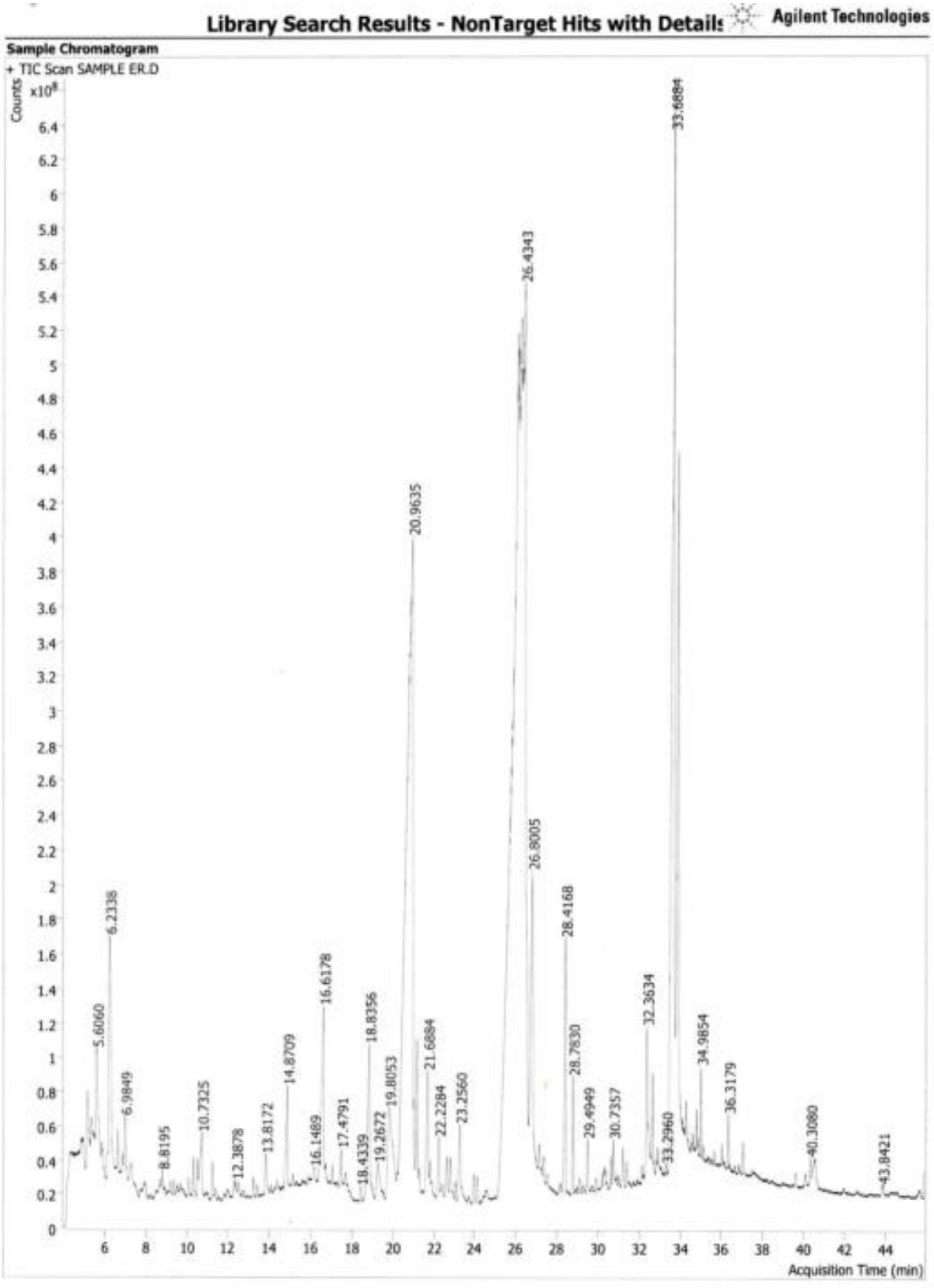
Appendix XIII: Chromatograms of the anthelmintic extracts



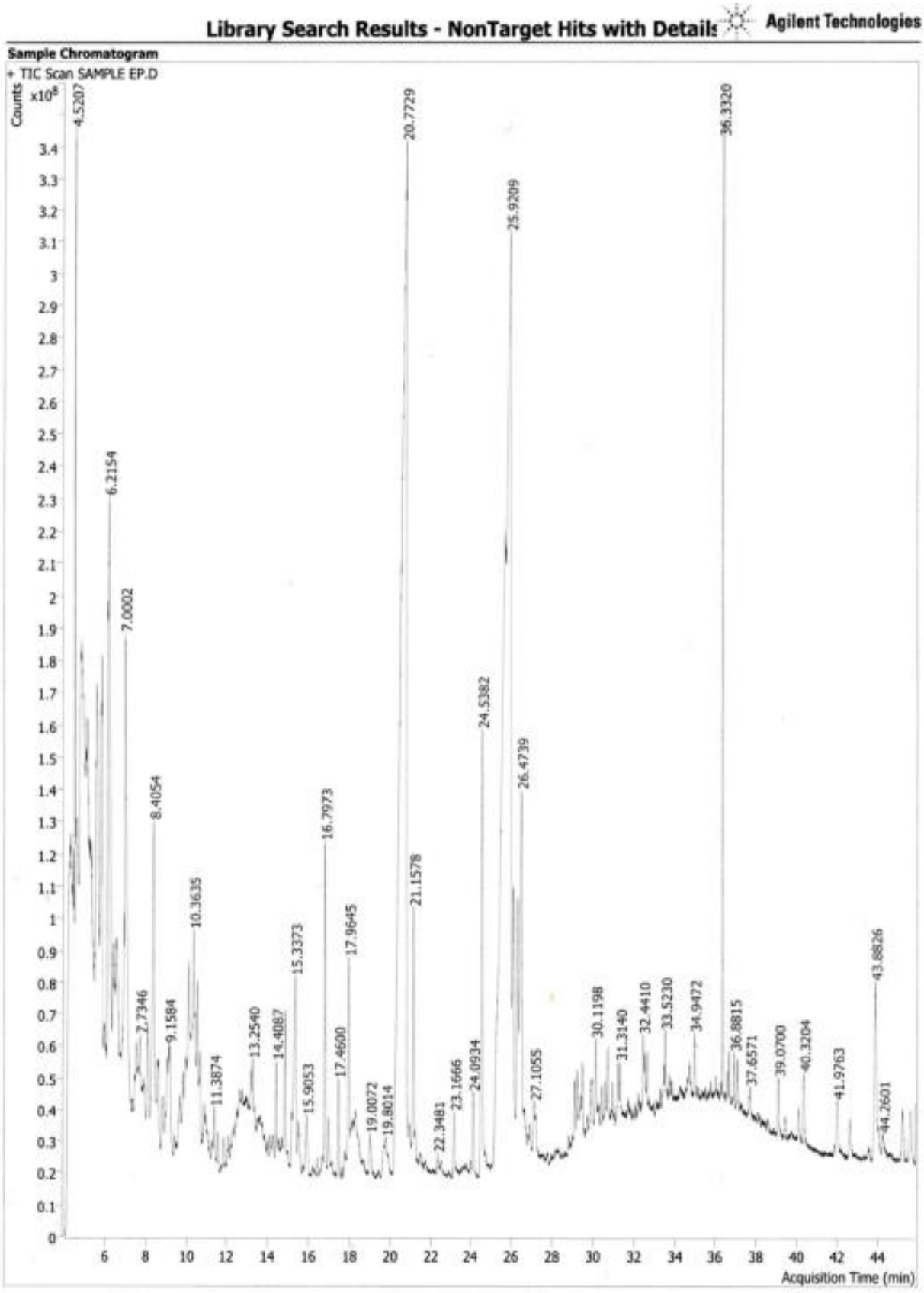
Chromatogram for the *Carica papaya* L. acetone extract



Chromatogram for the *Capsicum annuum* L. acetone extract



Chromatogram of *Capsicum annuum* L. ethanol extract



Chromatogram of *Carica papaya* L. ethanol extract