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RESEARCH

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Extracts of *Carica papaya* L. and *Capsicum annuum* L. showed comparable efficacy to piperazine citrate and levamisole hydrochloride in treatment of poultry helminths

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Abstract

Background In rural smallholder poultry production systems, synthetic anthelmintic drugs are considered expensive and in some instances ineffective because of anthelmintic resistance. We report on the phytochemical properties and efficacy of crude extracts of *Carica papaya* L. and *Capsicum annuum* L. against helminth infections of chickens. The experiments that compared the extract action to piperazine and levamisole were carried out in Soroti District, Eastern Uganda.

Method An experiment was set to evaluate efficacy of crude extracts of *C. papaya* and *C. annuum* against natural poultry helminths infections. Commercially available formulations of levamisole and piperazine were used to make a comparative efficacy study. Faecal egg count reduction (FECR) tests were used to measure efficacy of the treatments.

Results On gas chromatograph mass spectrometry (GC–MS) analysis of CPLa showed, vitamin C (42%), sterols (13%) and Triterpenoids (6%). CPLe contained lipids (45.04%), pyranones (20.3%), diterpenoids (4.9%), triterpenoids (3.5%), phenolics (3.1%), glycosides (2.2%) and steroids (1.4%). GC–MS analysis of CAFa gave lipids (45.04%), alkanes (27.7%) and alkaloids (8.2%). CAFe showed lipids (50.16%), alkaloids (22.73%), glycosides (3.61%) and pyranones (3.55%). In the in vitro assays, 0.08 g/ml of each of the extracts caused motility inhibition of more than 50% of adult *A. galli* after 5 h. The ranking of the in vivo average FECR was levamisole hydrochloride > CPLa > CAFa > CAFe > CPLe > piperazine citrate with the percentage reductions of 98.67 ± 2.309, 97.67 ± 2.517, 79.67 ± 1.528, 76.33 ± 1.528, 54.00 ± 2.00, 35.67 ± 2.082, respectively.

Conclusion The GC–MS analysis of the analysed plants shows presence of terpenoids, phenolics and alkaloids which are known for anthelmintic action. All the extracts caused higher FECR than piperazine. The presence of vitamin C in CPLa made it the best extract. Combinations of anthelmintics with vitamin C are recommended and toxicological studies of extracts.

Keywords *Capsicum annuum*, *Carica papaya*, Frequency of citation, Gas chromatography, Mass spectrometry, Worms, Vitamin C

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1 Introduction

Medicinal plants are widely used in farming communities across Africa and Asia for the treatment and control of poultry helminth infections [1–4]. The use of natural remedies (ethnoveterinary medicine) is regarded an indigenous technical knowledge in communities [5]. As such, little scientific research is dedicated to exploring and standardizing the use of natural products in the treatment and/or control of animal diseases [6]. Other than for cultural reasons, animal keepers, especially in developing countries, often resort to use of natural products because commercially available drugs are considered expensive [7]. Conventional commercial preparations may also require administration by a qualified technical person such as a veterinarian; seen as an additional cost to the poultry farmer [8]. Ethnoveterinary medicine is becoming popular because of the need of decreasing residues of synthetic drugs and the demand for organic food products [9]. Additionally, there are reports of widespread helminth resistance against common commercial anthelmintic products, hence the need for suitable alternatives [10]. Furthermore, there is an increasing campaign to promote consumption of residue-free organic foods, including animal products without anthelminthic residues [11]. Therefore, natural remedies, including safe and efficacious medicinal plants, are today seen as a viable alternative to conventional anthelmintic drugs. The use of ethnoveterinary practices (EVP) is getting popular in the different parts of the world. Use of EVP is becoming popular even in the high income countries like UK [12] and rural South Africa [13]. India has a rich ethnoveterinary base [14] while China has vast ethnoveterinary experiences which have been improved overtime [15]. In Uganda, at least 80% of farmers use medicinal plants in the treatment and control of poultry diseases, including helminth parasites [16]. The freshly collected plant material is usually crushed into a paste, mixed in water and orally administered [13]. The range of plants used and the practices in application are diverse. Specifically, capsicum and papaya plants are reportedly used widely in ethnoveterinary practice, including for poultry helminth control [17].

Helminths cause production losses and predispose poultry to microbial infections [18–21]. Helminths infections also reduce vaccine responses and can affect the general immune system of chicken. However, in mild and subclinical cases the signs of helminthiasis may not be observed [22]. Simultaneous presence of *Heterakis gallinarum* and *Histomonas meleagridis* in chickens increases Th1 cells and decreases splenic CD4 + cells [23]. Some helminths affect the gastrointestinal tract resulting into poor digestion and reduced absorption of food [24]. Helminths migrations lead to mechanical damages of various organs and cause stress to the host [25]. Clinical helminths infestations cause unthriftness, diarrhoea, inappetence and stuntedness among chicken [26]. *Syngamus trachea* causes respiratory distress. *Heterakis gallinarum* hosts *Histomonas meleagridis* which causes histomoniasis (typhllo-hepatitis) [27]. *Ascaridia galli* is very large nematodes that lead to intestinal obstructions especially in chicks. *Ascaridia galli* develops various associations with bacteria resulting in tissue damages and mortalities to chicken [28, 29]. Phenothiazine and piperazine combinations which were effective against *Heterakis gallinarum*, *Ascaridia galli* and tapeworms were banned by the Food and Drug Agency (FDA) of the USA in 2004 [30].

Carica papaya L. tree is a giant tropical herb with a semi-woody usually single stem growing to about 10 m high [31]. The leaves are about 60 cm wide with deeply palmated lobes. The leaves are attached to long hollow stalks [32]. *Capsicum annuum* L. is tropical perennial shrubs of the family Solanaceae. *Carica papaya* L. plant parts are mentioned as promising against helminths generally [33]. Various plants of the genus *Capsicum* have been found to be effective alternative against helminths in different animals [34, 35]. The fruits are red and taper gradually being pointed at the end. The choice of plants was informed by our earlier work on ethnoveterinary practices [17, 36]. There is limited published information on phytochemical composition and effectiveness of these products. We, thus, present results of phytochemical composition, in vitro *and* in vivo experiments to test the efficacy of *Capsicum annuum* L. and *Carica papaya* L. extracts against poultry helminths.

2 Materials and methods

2.1 Description of study area

The study was in Soroti district (Fig. 1). Soroti district is located at Latitude $1^{0}42^{I}$ 47.4516^{II}N and Longitude $33^{0}36^{I}$ 22.986^{II}E. It is one of the areas with a high number of households keeping chickens in Eastern Uganda [37].

2.2 Study design

This was a controlled experiment designed initially to test in vitro efficacy of crude extracts of *Carica papaya* L. and *capsicum annuum* L. using piperazine citrate as the positive control. The extracts were further tested in live chickens randomly assigned to different experimental treatments including levamisole hydrochloride and piperazine citrate. The study followed a survey on plants used against chicken helminths [36].

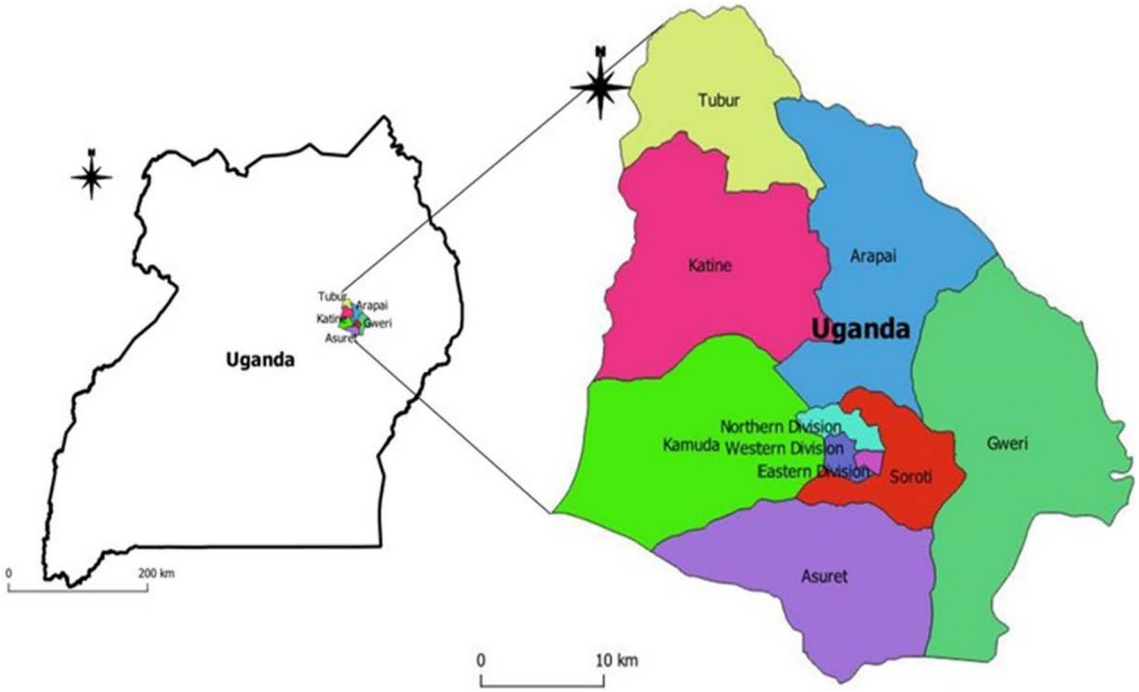

Fig. 1 Map showing the subcounties of Soroti district of Uganda, adopted from [36]

2.3 Description and care of experimental chicken

A total of 100 one-day-old indigenous chickens were raised free range with mother hens and offered night shelters. The chicks scavenged with the mother hens, and no treatments were administered except the Newcastle virus vaccination. They were purposively left to acquire helminths naturally during feeding. They were selected for experiments in the 7th week (Fig. 2).

2.4 Preparation of acetone and ethanolic extracts

Fresh leaves of *Carica papaya* (pawpaw) and ripened fruits of *Capsicum annuum* (bird's eye chilli) were collected from homesteads in Soroti district between May–June, 2024. The collected plant materials were washed with tap water to remove observable debris and air dried under shade on the farm of the Faculty of Agriculture and Animal Sciences, Busitema University, for two weeks. Using a coffee grinder (silver crest®) model SC-1880, from Guangzhou China, the dried plant materials were ground into fine powder. Extracts were obtained

Fig. 2 The structure in Arapai—Busitema University where the experimental chickens were kept

using the conventional Soxhlet method [38] with minor modifications. Ten grams of ground plant material with 5g of pumice stone were placed in a cellulose thimble plugged with cotton. Extraction was performed using a solid-to-liquid ratio of 1–12 (g/ml) for 8 h. The extraction was done in duplicate using analytical grade ethanol and acetone as solvents. The *Carica papaya* L. ethanol extract was labelled CPLe and the acetone extract labelled CPLa. The *capsicum annuum* L. ethanol extract was labelled CAFe and the acetone extract labelled CAFa. The extract was concentrated under vacuum at 40 °C and kept at 4 °C pending use.

2.5 Phytochemical and GC_MS analysis of plant extracts

Phytochemical analysis of acetone extracts was done according to the methods of Harborne [39]. Phytochemical analysis of ethanol extracts was done according to the methods of Ejikeme et al. [40], Rao et al. [41], Chauke et al. [42], Sorescu et al.[43], Sankhalkar and Verneka [44]. For GC–MS analysis, dried extract was dissolved in 50µL moxifloxacin (Mox) and held at 37 °C for 90 min. The derivatization was initiated by adding 50µL N-methyl-N-tert-butyldimethylsilyltrifluoroacetamide (MTBSTFA) and 1% tert-butyldimethylsilyl (TBDMS) followed by incubation at 55 °C for 60 min. After centrifugation at 13,000 rpm for 10 min, the supernatant was collected and analysed by GC–MS.

GC–MS (GC–MS- agilent), model 7000D triple quadruplets equipped with a split injector (Split ratio 1:0) was used. The injection temperature setting was at 250 °C, and the injected volume was 1 µL. The column (ZB-5SMi, 30 m × 0.25 mm × 0.25 µm) was used. The column temperature programme 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 °C, and 15 min to 310 °C. Helium was employed as the carrier gas at an average linear velocity of 44 0.5 cm/sec, prime pressure of 500–900. The flow control mode had pressure at 99.8 kPa, total flow (50 mL/min), column flow (1.46 ml/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.

2.6 In vitro susceptibility tests

Naturally infected local breed chicks of 7–8 weeks described in Sect. 2.3 were used as the source of helminths. The birds were sacrificed and the *Ascaridia galli* collected from the intestines (Fig. 7). *Ascaridia galli* were chosen because they are the commonest chicken worms, are easily seen and are not fragile. The *Ascaridia galli* from intestines of chicken was washed in PBS (0.01 M PBS, 0.138 M NaCl, 0.0027 M 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 (10 ml) of the extracts were poured in the petri dishes and 10 worms were added to each petri dish. 0.025 g/ml 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 30 min for 300 min.

2.7 In vivo efficacy experiment

The chicken for the experiment were selected from those described in Sect. 2.3. On the day of recruitment, each chicken was kept in isolation for between 30 and 60 min or until it voided a faecal dropping. The faecal dropping was then subjected to helminth egg identification and counting as described by Glennon [45]. Chickens that had stool with helminth egg counts above 200 epg were selected for experimental treatments. Selected chickens each weighing about 300–350 g were put in cages and kept inside a well ventilated poultry house (Fig. 2). At least three experimental chickens were kept in each cage (0.16 m^2). Commercial growers mash (Nuvita®) was fed with each bird receiving about 55 g of the mash daily. Tap water was given ad libitum. The birds were allowed to acclimatize in this condition for seven days prior to experimental treatments. Each chicken was forcefully administered with 3 ml (0.48 g) of the constituted extract in the mouth for the extracts groups using a syringe; levamisole hydrochloride was given at 25 mg/kg and piperazine citrate at 100 mg/kg. The concentration of the plant extracts was determined from previous in vitro experiments as double the lowest concentration that inhibited motility of the highest number of *A. galli* worms, this was done to simulate the discriminating concentration concept [46]. The lowest in vitro concentration that inhibited motility of more than half of the mature *A. galli* was 0.08 g/ml of the extract for most extracts; it was doubled to 0.16 g/ml in vivo concentration and the birds received 0.48 g for each day. The treatment was repeated on the second day.

A week after treatments, 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. Egg counts were determined by modified McMaster technique [41].

2.8 Statistical analysis

Phytochemical percentage compositions were tabulated in Microsoft Excel and presented in tables. In vitro data of number of non-motile *Ascaridia galli* (number of

worms that could not move or turn their bodies when tapped with a small metal rod) were entered in Excel in triplicates and transferred to SPSS version 26. One-way ANOVA was used to determine any significant difference between the means of number of non-motile *Ascaridia galli* per treatment. Tukey's HSD was used to determine the specific treatment groups that were different from each other. The in vivo data of ECG were entered in Excel in triplicates and transferred to SPSS version 26. One-way ANOVA was used to determine any significant difference between the means of ECG per treatment. Tukey's HSD was used to determine the specific treatment groups that differed from each other. The differences were considered significant when $p \leq 0.05$.

2.9 Ethical considerations

An institutional ethical review certificate was acquired from the school of Veterinary Medicine and Animal Resources, College of Veterinary Medicine and Biosecurity, Makerere University.

The permission to collect plant materials which were both wild and planted was granted by the Uganda National Council of Science and Technology (UNCST) under permission number- A220ES. The guidelines that were given by Institution Review Board and UNCST were followed, and no other licenses were required.

3 Results

3.1 Qualitative phytochemical analysis

The results are detailed in Table 1;

Table 1 Qualitative phytochemical analysis of *Carica papaya* L. and *Capsicum annuum* L

Compound	*Carica papaya* L. extract		*Capsicum annuum* L	
	(CPLe)	(CPLa)	(CAFe)	(CAFa)
Saponins	+	–	–	–
Tannins	–	–	–	–
Reducing compounds	+	–	+	–
Alkaloid salts	+	+	+	+
Anthocyanosides	–	–	+	–
Anthraconosides	–	–	+	–
Coumarins	+	+	+	+
Flavonosides	+	+	+	+
Steroid glycosides	+	+	+	+

None of the extracts showed presence of tannins

3.2 GC–MS profile of *Carica papaya* L. and *Capsicum annuum* L. extract

(CPLa) yielded vitamin C (42%), sterols (13%) and triterpenoids (6%) (Table 2 and Fig. 3). (CPLe) yielded sterols (33%), pyranones (20.3%), diterpenoids (4.9%), triterpenoids (3.5%) phenolics (3.1%), glycosides (2.2%) and steroids (1.4%) (Table 3 and Fig. 4). (CAFa) yielded sterols (45.04%), alkanes (27.7%) and alkaloids (8.2%) (Table 4 and Fig. 5). (CAFe) yielded sterols (50.16%), alkaloids (22.73%), glycosides (3.61%) and pyranones (3.55%) (Table 5 and Figs. 6, 7).

3.3 In vitro efficacy

3.3.1 Capsicum annuum L. extracts (CAFa & CAFe)

The extracts took longer to act when compared to piperazine citrate but showed anthelmintic activity. The extracts did not cause any observable lesions on the skin whatsoever. 4–5 h was needed for the extracts to make *Ascaridia galli* immotile. A concentration of 0.08 g/ ml made over 50% of the mature *A. galli* immotile after 5 h

Table 2 GC–MS profile of *Carica papaya* L. leaves acetone extract (CPLa)

Retention time	Compound name (AP)/Group of compound	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	$C_{19}H_{32}N_2O_3$	30,902,350.6	57	31
16.7910	9-Hexadecenoic acid, 9-octadecenyl ester, (z,z)- / (sterols)	22393-98-2	$C_{34}H_{64}O_2$	7,707,685.4	63.3	7.7
20.2233	1-(+)-Ascorbic acid 2,6-dihexadecanoate / (vitamin C)	28474-90-0	$C_{38}H_{68}O_8$	42,045,756.6	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]cyclounde-cen-11-yl ester, [1aR-[1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*(E),11aS*]]- /(sterols)	51906-13-9	$C_{27}H_{38}O_8$	5,309,888.5	61.2	5.3
29.0563	D:A-Friedooleanan-3-ol,(3,alpha.)-/(Triterpenoids)	5085-72-3	$C_{30}H_{52}O$	6,262,628.8	51.2	6

(CPLa) yielded 13% sterols and 6% triterpenoids which are some of the compounds with known anthelmintic action

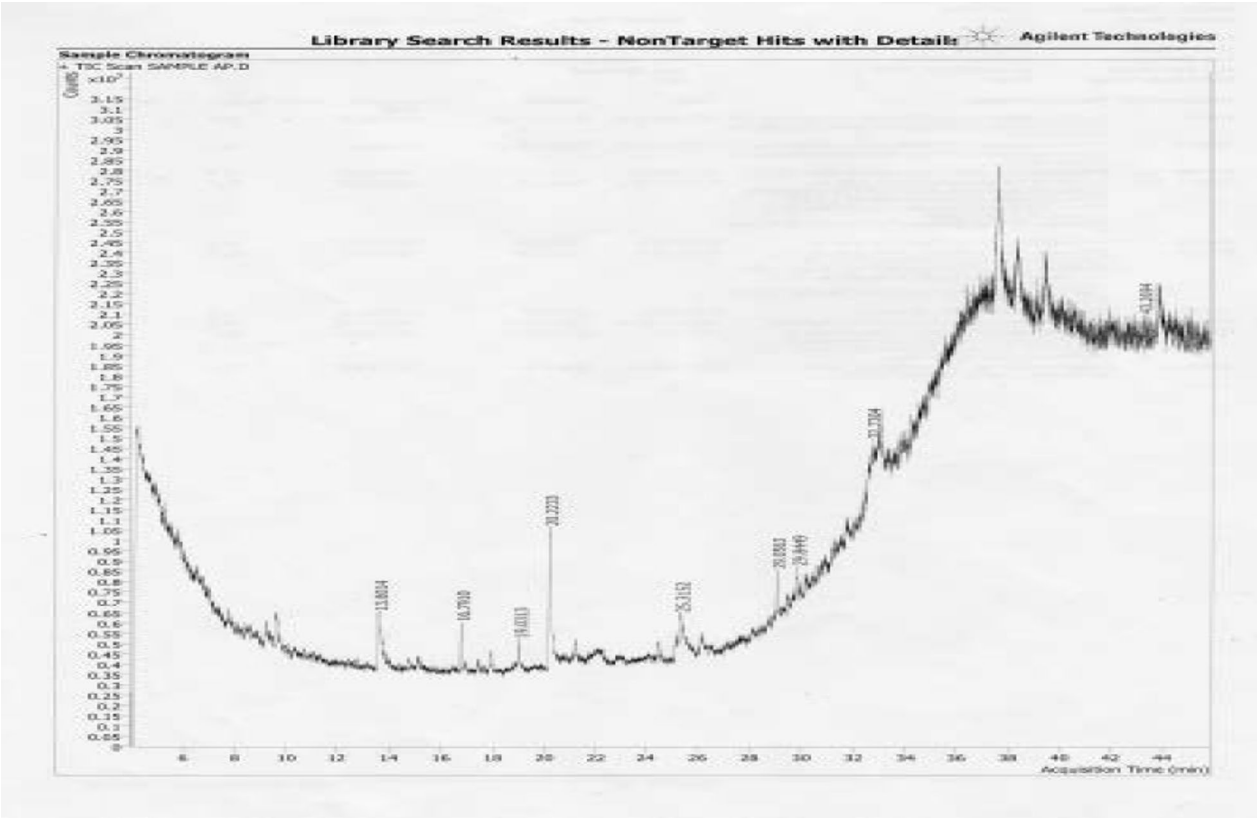

Fig. 3 Chromatogram of *Carica papaya* L. leaves acetone extract (CPLa)

Table 3 GC–MS profile of *Carica papaya* L. leaves ethanolic extract (CPLe)

Retention time	Compound Name (EP)/ Group of compound	CAS#	Formula	Component area	Match factor	Estimated conc. (%)
4.4683	Benzene,1,2- dichloro-	95-50-1	$C_6H_4Cl_2$	310,372,634.1	77.4	1.5
4.8495	Benzyl alcohol	100-51-6	C_7H_8O	656,899,632	74.3	3.1
5.5951	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-/(Pyranones)	28564-83-2	$C_6H_8O_4$	4,244,572,882.5	79.7	20.3
7.0002	Phenol, 5-ethenyl-2methoxy-/(Phenolics)	621-58-9	$C_9H_{10}O_2$	652,974,980.4	81.6	3.1
8.4054	Ethyl beta-d-riboside/(glycosides)	1000126-95-4	$C_7H_{14}O_5$	470,071,693.6	70.8	2.2
16.7973	Neophytadiene/(diterpenoids)	504-96-1	$C_{20}H_{38}$	341,714,834.0	87.2	1.6
20.6459	n-Hexadecanoic acid/(sterols)	57-10-3	$C_{16}H_{32}O_2$	1,352,846,816.3	64.9	6.5
21.1578	Hexadecanoic acid, ethyl ester/(sterols)	628-97-7	$C_{18}H_{36}O_2$	242,270,004.7	83.5	1.2
24.5382	Phytol/(diterpenoids)	150-86-7	$C_{20}H_{40}O$	699,650,422.6	88.3	3.3
25.6724	9,12,15-Octadecatrienoic acid (Z,Z,Z)-/(sterols)	463-40-1	$C_{18}H_{30}O_2$	4,364,890,955.8	87.4	20.8
26.4739	Octadecanoic acid/(sterols)	57-11-4	$C_{18}H_{36}O_2$	639,975,153.6	84.3	3.1
36.3320	Supraene/(Triterpenoids)	7683-64-9	$C_{30}H_{50}$	731,801,424.0	84.5	3.5
43.8826	beta.-Sitosterol/(Steroids)	83-46-5	$C_{29}H_{50}O$	284,103,679.4	81.2	1.4

(CPLe) yielded 25.3% sterols, 8.4% terpenoids and 3.1% phenolics which are some of the compounds with known anthelmintic action

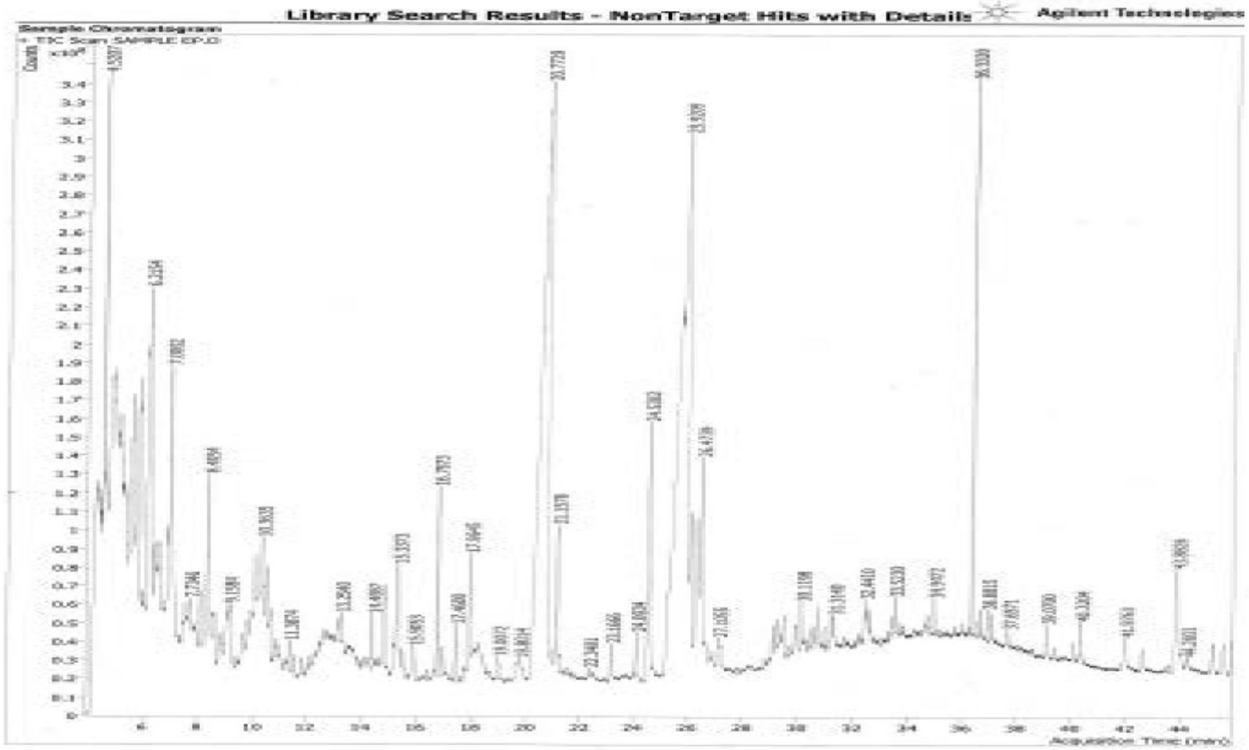

Fig. 4 Chromatogram of *Carica papaya* L. leaves ethanolic extract (CPLe)

Table 4 GC–MS profile of *Capsicum annuum* L. fruits acetone extract (CAFa)

Retention time	Compound Name (AR)/ Group of compound	CAS#	Formula	Component area	Match factor	Estimated conc. (%)
25.8299	9,12-Octadecadienoic acid (Z,Z)-/(sterols)	60-33-3	$C_{18}H_{32}O_2$	1,344,761,417.7	88.4	9.91
26.0223	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-/(sterols)	463-40-1	$C_{18}H_{30}O_2$	275,995,441.8	60.1	2.03
26.4819	Pentadecanoic acid/(sterols)	1002-84-2	$C_{15}H_{30}O_2$	546,092,918.2	83.6	4.02
32.5972	Heptacosane/(alkanes)	593-49-7	$C_{27}H_{56}$	1,745,180,778	81.2	12.86
33.4436	Capsaicin/(Alkaloid)	404-86-4	$C_{18}H_{27}NO_3$	802,053,360.9	84.9	5.91
33.6958	Dihydrocapsaicin/(Alkaloids)	19408-84-5	$C_{18}H_{29}NO_3$	311,451,357.4	72.5	2.29
34.9720	Heneicosane/(alkane)	629-94-7	$C_{21}H_{44}$	748,932,682.8	84.2	5.52
36.3398	Squalene/(sterols)	111-02-4	$C_{30}H_{50}$	3,032,372,293.2	85.0	22.34
36.8537	1-Heptacosanol/(sterol-alcohol)	2004-39-9	$C_{27}H_{56}O$	384,232,719.6	85.3	2.83
38.1879	Hentriacontane/(alkane)	630-04-6	$C_{31}H_{64}$	1,264,907,160	76.5	9.32
39.3970	1-Heptacosanol/(sterols)	2004-39-9	$C_{27}H_{56}O$	252,395,509.5	83.7	1.86
41.2582	Tetratetracontane/(sterols)	7098-22-8	$C_{44}H_{90}$	278,416,640.1	71.4	2.05

(CAFa) yielded 45.04% sterols and 8.2% alkaloids which are some of the compounds with known anthelmintic action

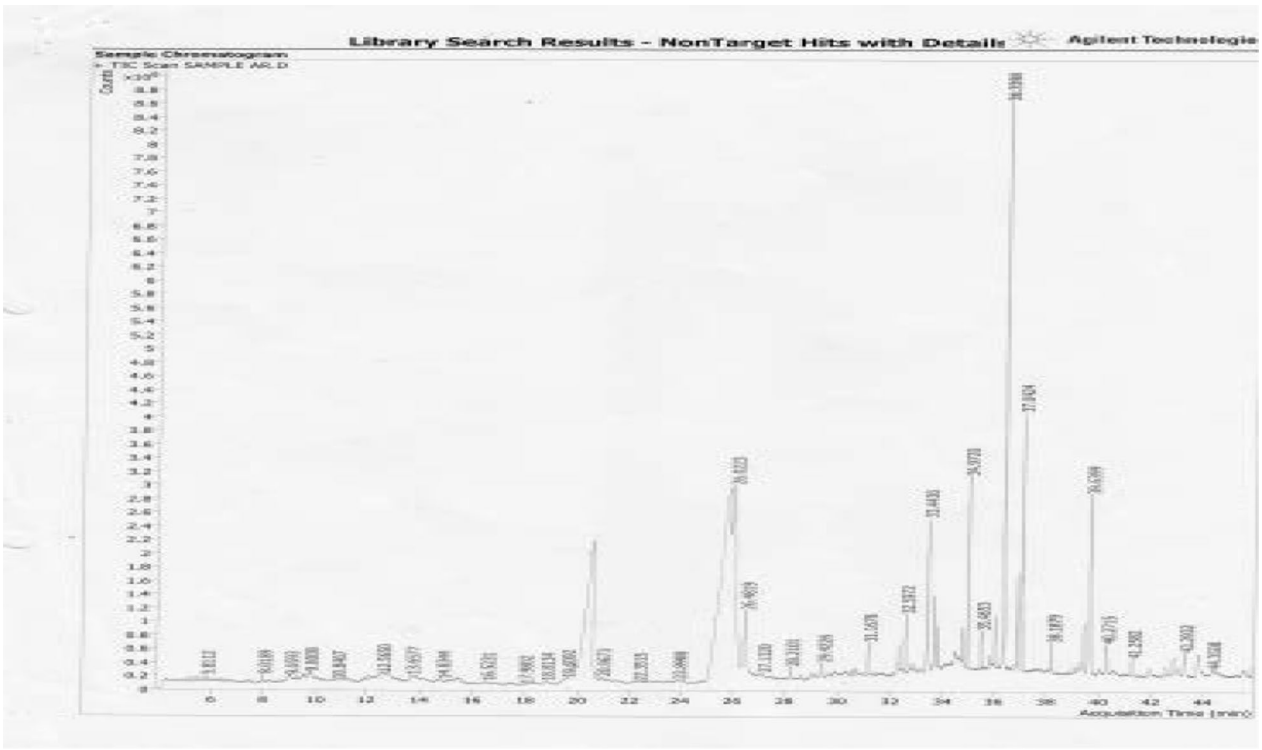

Fig. 5 Chromatogram of *Capsicum annuum* L. fruits acetone extract (CAFa)

Table 5 GC–MS profile of *Capsicum annuum* L. ethanolic extract (CAFe)

Retention time	Compound Name (ER)/Group of compound	CAS#	Formula	Component area	Match factor	Estimated conc. (%)
6.2338	5-Hydroxymethylfurfural/(glycosides)	67-47-0	$C_6H_6O_3$	1,039,557,851.9	81.7	3.61
16.1489	Oleic acid/(sterol)	112-80-1	$C_{18}H_{34}O_2$	1,263,336,730	68.9	4.39
16.6178	Pentadecanoic acid/(sterol)	1002-84-2	$C_{15}H_{30}O_2$	806,683,607.3	86.5	2.80
18.8356	Palmitoleic acid/(sterol)	373-49-9	$C_{16}H_{30}O_2$	1,194,629,505	84.0	4.15
19.2672	n-Hexadecanoic acid/(sterol)	57-10-3	$C_{16}H_{32}O_2$	1,090,877,057	73.3	3.79
21.6884	Cic-10-Heptadecenoic acid/(sterol)	29743-97-3	$C_{17}H_{32}O_2$	602,677,070.9	81.7	2.09
26.0962	Ethanol, 2-(9,12-octadecadienyloxy)-(Z,Z)-	17367-08-7	$C_{20}H_{38}O_2$	1,539,700,382	80.6	5.35
26.3316	2H-Pyran-2-one, tetrahydro-6-tridecyl-/(pyranones)	1227-51-6	$C_{18}H_{34}O_2$	1,021,059,290.5	55.2	3.55
26.4343	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-/(sterol)	463-40-1	$C_{18}H_{30}O_2$	2,107,179,713.2	67.2	7.32
26.8005	Octadecanoic acid/(sterol)	57-11-4	$C_{18}H_{36}O_2$	942,150,689.0	83.3	3.27
28.4168	Arachidamide, N-methyl-/(sterol)	1000420-44-0	$C_{21}H_{43}NO$	6,433,662,652.3	64.0	22.35
31.4010	Capsaicin/(Alkaloid)	404-86-4	$C_{18}H_{27}NO_3$	4,834,637,352	65.6	16.80
33.9070	Dihydrocapsaicin/(Alkaloid)	19408-84-5	$C_{18}H_{29}NO_3$	1,706,326,041	78.9	5.93

(CAFe) yielded 50.09% sterols and 22.73% alkaloids which are some of the compounds with known anthelmintic action

while 0.32 g/mL made over 80% mature *A. galli* immotile, Table 6.

3.3.2 Carica papaya L. extracts

CPLe acted on *A. galli* faster than *Capsicum annuum* L.

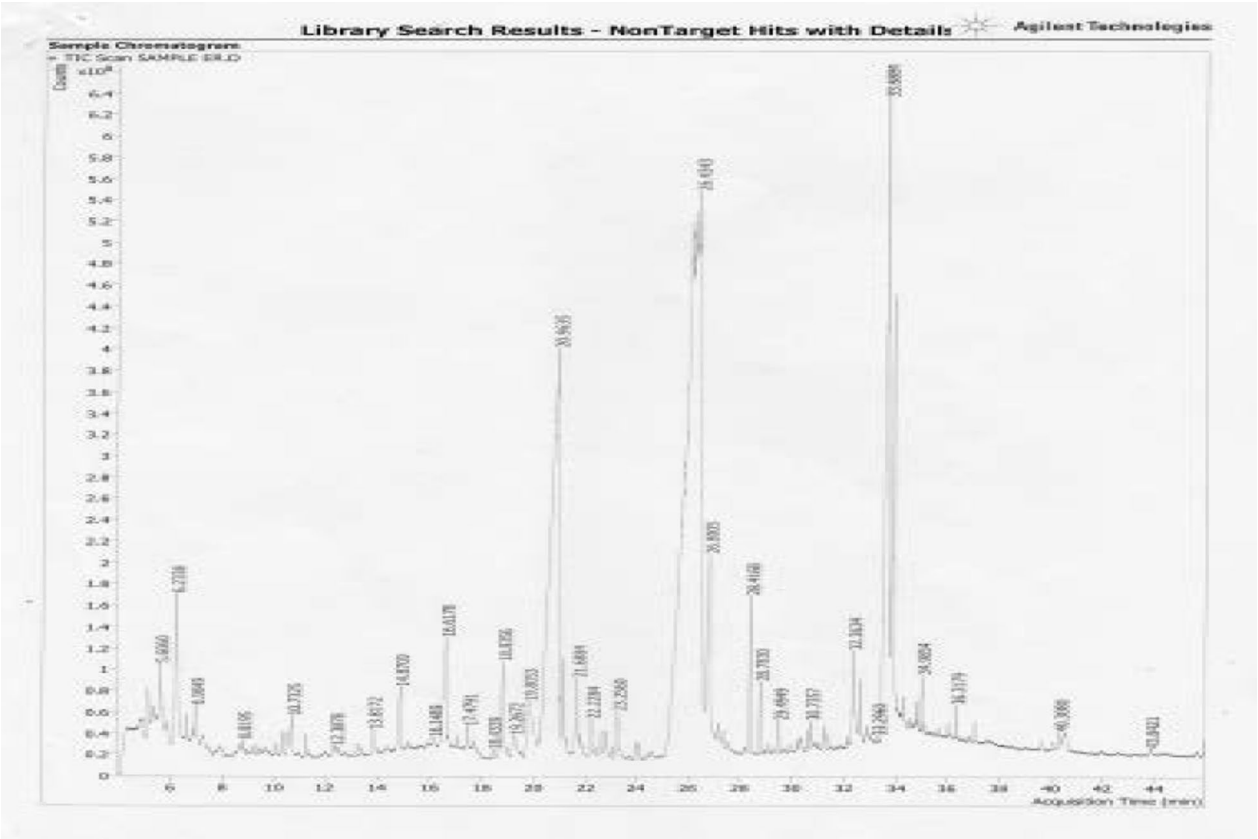

Fig. 6 Chromatogram of *Capsicum annuum* L. ethanolic extract (CAFe

fruit extracts. After 240 min 0.32 g/ml CPLe made 75% of adult *A. galli* immotile. After 270 min, 0.08 g/ml had made 50% of adult *A. galli* immotile, and by 300 min, 0.08 g/ml had made 65% of the adult *A. galli*. immotile, Table 6.

CPLa by 240 min, 0.08 g/ml had made 55% of adult *A. galli* immotile and 0.32 g/ml had made 100% of adult *A. galli* immotile. By 300 min, 0.08 g/ml had made 70% of adult *A. galli* immotile, Table 6.

3.4 In vivo efficacy

All extracts were significantly more effective compared to PBS ($p=0.000$), and CPLa was not significantly different from levamisole (Tables 7, 8)

$$\%\,\text{FEC reduction test(FECR)} = \frac{100(\text{FEC control} - \text{FEC treated})}{\text{FEC control}}$$

4 Discussions

4.1 Qualitative phytochemical analysis of *Carica papaya* L. and *Capsicum annuum* L.

The phytochemicals studied in this work were those commonly known to have anthelmintic activity; saponins have been reported to have anthelmintic effects [47, 48]. Alkaloids are also reported to have action against helminths [49, 50]. Coumarins have inhibitory effects against helminths [51, 52]. Some flavonoids are effective against helminths [53, 54]. Some steroids are effective against helminths [55].

4.2 Quantitative phytochemical analysis of *Carica papaya* L. and *Capsicum annuum* L.

Ethanoic extracts (CPLe and CAFe) had more compounds than acetone extracts (CPLa and CAFa). CPLa had the best in vivo action; this opportunity can be utilized at industrial level when scaling up production. Steroids are said to have anthelmintic effects [5] but the action of specific lipids on helminths is not fully known.

Fig. 7 Harvesting of adult *A. galli* from chicken

Vitamin C does not have particular action against helminths but it improves the response of hosts to helminths infections [56]. Triterpenoids have known effects on helminths [57, 58]; Saponins (triterpenoids, steroids) inhibit mitochondrial action and affect helminth cell membrane damaging helminths [59]. Terpenoids inhibit neurotransmission causing paralysis worms, and they also inhibit hatching of worm eggs [60]. 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 is not known and requires further investigation. Phenolics have known action against helminths [61–66]; however, the particular effect of Phenol, 5-ethenyl-2methoxy- on helminths is not known. Particular glycosides especially flavonal glycosides have anthelmintic action [67]; however, the action of all other types of glycosides against helminths requires further research. Diterpenoids have known action against helminths [68]. Phytol is known to be effective against helminths [69], but the effect of Neophytadiene on helminths requires further research. The action of alkanes against helminths is not known and requires further research. Alkaloids have known action against helminths [48, 70]; Capsaicin and Dihydrocapsaicin are known to be effective against helminths especially due to their pungency action [71]. Alkaloids are neurotoxic to helminths [72].

4.2.1 In vitro efficacy tests of the extracts

No deaths were observed nor any observable lesions on the *A. galli* dermis; however, motility inhibition was observed 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. [68] and Sugiharto [69]. The findings regarding *Capsicum annuum* L. extracts are in

Table 6 In vitro efficacy tests of the extracts, positive control (*Piperazine citrate*) and negative control (PBS)

Treatment	Concentration g/mL	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min
CAFe	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
	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
CAFa	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
	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
CPLe	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
	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
CPLa	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
	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

The mean number of immobilized *A. galli* for each concentration after the specified number of minutes, (±) standard deviation

Table 7 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 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	13,800	12,750	13,120	0	0	0	0.00 ± 0.000

Egg counts per gram of faeces (ecg), faecal egg count reductions (FECR) and mean FECR. Although CAFa and CAFe were effective, CPLa was the most effective, almost as good as levamisole

agreement with Gentiles et al. [34] who reported the high anthelmintic potency of *Capsicum annuum* var. Longum.

No major difference in the action for CAFa and CAFe because they had almost the same composition of the known phytochemical compounds.

The CPLa caused faster motility inhibition compared to 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. The findings are in agreement with Sen et al. [70] who found 100% in vitro effect against *A. galli* at even 20 mg/ml; he also showed that the extracts were slower and required longer time periods (5–7 h). The findings are also in agreement with Cabral et al., [71] who achieved 100% in vitro action against *Strongyloides stercoralis* using 566 mg/ml of *Carica papaya* L. extracts. Increasing the concentration several folds reduced the action time in the in vitro assays.

Table 8 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

4.3 In vivo efficacy tests of the extracts, piperazine and levamisole

All extracts were effective because they caused above 50% faecal egg count reductions; however, the *Carica papaya* L. extracts were superior to those of *Capsicum annuum* L. CPLa extracts were distinctly superior to CPLe in faecal egg reductions. The superior action was attributed to vitamin C in presence with other anthelmintic compounds. The action of vitamin C on helminths in the presence of various types of anthelmintics is not well known although Sengupta et al., [73] mention that the observed actions arise from vitamin C enhancing host defences. The findings about the in vivo efficacy of *Carica papaya* L. extracts are in agreement with Sen et al. [70]. The in vivo efficacy of *Capsicum annuum* L. extracts are in agreement with Gentiles et al. [34] who observed significant faecal egg reductions.

CPLa was as effective as levamisole, was different from CPLe; CPLa had higher concentration of vitamin C. There is need to investigate how vitamin C changes the action of synthetic and herbal anthelmintics. All extracts had higher FECR than piperazine citrate but levamisole hydrochloride had 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. Further studies on resistance are recommended to confirm these findings. Acetone gave better performing extracts than the more polar ethanol; chicken farmers could benefit from less polar acetone compared to their polar aqueous solvents. However, acetone extracts are immiscible with water, require the use of 2% DMSO to mix; the procedures which can be achieved in the laboratory and not by farmers. More research on the extracts of less polar and non-polar solvents is advised to guide the scaling of these anthelmintic alternatives. Producing of extracts for wider use requires funding to enable commercial farming of the medicinal plants, further research on efficacies and investing in machinery for processing plant anthelmintics. Wider use of plant anthelmintics requires fractionating, structural elucidations and increased awareness through research dissemination and publications. However, toxicity studies and testing products across species for animal species requirement should precede plans of scaling up.

4.4 Future work

Pyranones were in relatively high concentrations in both *Capsicum annuum* L. and *Carica papaya* L.; it is

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.

4.5 Research limitations

1. Only two solvents were used in the extraction (ethanol and acetone). More solvents could have increased the types of extracts even the phytochemicals for a more comprehensive evaluation of the anthelmintic plants.
2. In vitro assays used only *Ascaridia galli*, and the responses of other chicken helminths were assumed to be like for *Ascaridia galli.* Other helminths are fragile or can only be clearly seen with aided eyes.
3. Adult fresh *Ascaridia galli* was used in the in vitro assays; the responses of other stages are not known. In vitro responses of other *Ascaridia galli* stages were not studied.
4. Only the indigenous chickens were used.
5. The phytochemical analysis was done only during the rainy season when the plants can easily be found. The extracts composition during the dry season is not known.

Abbreviations

CD	Cell differentiation
DRC	Democratic republic of Congo
EVP	Ethnoveterinary practice
FAO	Food and Agricultural Organization
FDA	Food and Agriculture Organization
GC–MS	Gas chromatography–mass spectrometry
CPLa	*Carica papaya* Leaf acetone extract
CPLe	Carica papaya leaf ethanol extract
CAFa	*Capsicum annuum* Fruit acetone extract
Cafe	*Capsicum annul* Fruit ethanol extract
FECR	Faecal egg count reduction
SPP	Species
UK	United Kingdom
m	Metres
g	Grams
PBS	Phosphate-buffered saline
ml	Millilitres
^{o}C	Degrees centigrade
ANOVA	Analysis of Variance
HSD	Honestly significant difference
ECG	Egg count per gram
SPSS	Statistical Package for Social Sciences
min	Minutes
µm	Micrometres
µL	Microlitres

Supplementary Information

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Additional file 1.

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Author contributions
GZ conceptualized the research; JK, SB and JOA supervised the conducting of the field work of data collection. GZ conducted the field work, processed the required permissions. GZ, JK, SB and JOA sourced the funding while all the members participated in making the first draft manuscript. All members (GZ, JK, SN, PV, FO, SB and JOA) participated in polishing the article for submission.

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Availability of data and materials
The datasets supporting the conclusion of this article are included with in the article and its additional files. Data are provided within the manuscript or supplementary information files.

Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
An institutional ethical review certificate was acquired from the School of Veterinary Medicine and Animal Resources, College of Veterinary Medicine and Biosecurity, Makerere University. The permission to collect plant materials which were both wild and planted was granted by the Uganda National Council of Science and Technology (UNCST) under permission number-A220ES. The guidelines that were given by the Institution Review Board and UNCST were followed, and no other licenses were required.

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