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Chapter 12

The *in vitro* Antihelminthic Efficacy of *Erythrina Abyssinica* Extracts on *Ascaridia galli*

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Additional information is available at the end of the chapter

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1. Introduction

Helminth Infestation can lead to reduced growth and egg production in poultry. Coupled with high costs in 'wasted feeding' and demand for de-worming, this result in considerable economic losses in poultry enterprises and directly affects livelihood of small holder farmers [1]. In the birds, there is slow growth rate hence reduced body weight, delayed market weight attainment because of competition for nutrients by the bird and parasites. For the farmer, there is loss of income, reduced employment, and compromised household welfare, difficulty to raise educational fees, health fess, and social security activities.

Though an important veterinary practice, helminths control is largely neglected in village-level chicken production. The situation demands for alternative and inexpensive helminths control measures. Locally available medicinal plants have traditionally been used by small holder farmers to manage various livestock and human ailments [1-4], However, scientific data on the efficacy of these plants in helminthic control is lacking [5-8], It has been reported that local communities in the south-western agro-ecological zone (SWAEZ), Uganda, use *Erythrina abyssinica* (Leguminocae) extracts to deworm village chicken [9].

Ascaridiasis is a common disease of poultry (especially chicken and turkeys) in Uganda; it's caused by a nematode, *Ascaridia galli*. *A. galli* is a highly pathogenic worm residing in small intestines and is transmitted through eggs.

Ascaridiasis lead to weight depression and in severe cases causes intestinal blockage, loss of blood, reduced sugar content, retarded growth and mortality. It was noted that the age of the host and severity of exposure play a role in *A. galli* infections. Chickens older than 3-months are largely resistant to *A. galli* infection. *A galli* larvae undergo little to no development in older chicken. Larval development is arrested in the third stage at high dose rates as a result of resistance

rather than a density-dependent phenomenon. Also, heavier broiler breeds are known to be more resistant to Ascarid infections than lighter white leghorn chicken [1,4],

The study hypothesized that plants with known but undocumented anthelmintic activity exist in the SWAEZ of Uganda. The efficacy of medicinal plant varies with the location in the SWAEZ. This study aimed to investigate the efficacy of *Erythrina abyssinica* under in vitro conditions and to compare its efficacy with that of a conventional drug, the piperazine citrate.

2. Materials and methods

a. Collection and maintenance of the worms

Ascarid worms have a large, thick, yellowish white head with 3 large lips. The male is 50-76 mm long, 490-1.21 mm wide. It has a preanal sucker oval or circular, with strong chitinous wall with a papilliform interruption on its posterior rim; tail with narrow caudal alae or membranes and 10 pairs of papillae. The female is 60-116 mm long, 900-1.8mm wide; the vulva is the anterior part of the body, and eggs are elliptical, thick Shelled and not embryonated at time of deposition [10].

The worms used in this study were collected from fresh intestines taken from slaughtered indigenous chicken from the Bwaise market. The intestines were grossly evaluated and the worms removed and preserved in Goodwin's solution at a temp of 37°c in an incubator.

The Goodwin's physiological solution was prepared as following [11,12,13,14, 15]. The constituents of Goodwin's physiological solution were calcium chloride (0.20g), glucose (5g), magnesium chloride (0.10g), potassium chloride (0.2g), sodium bicarbonate (0.15g), sodium chloride (8g) and sodium hydrogen phosphate (0.5g), all quantities dissolved in one (1) litre of distilled water. Calcium chloride was added later after dissolving other salts to discourage its precipitation. The solution was pre warmed to 37^0C before placing in the worms.

b. *Erythrina abysinnica* selection and extraction procedures

Erythrina abyssinica (local and Luganda names: *Muyigiti;* Runyakole name*:Ekiko*) (Figure 1), is a deciduous savannah species. It grows in open woodland and grassland. It has characteristic red overflowing flowers. It can be propagated through seedlings, cuttings and truncheons. In the south-western rangelands of Uganda, it is sometimes planted along fences of paddocks to support barbed wires. It has various traditional medicinal applications in livestock. It is also used in traditional human medicine [2, 14].

The leaves, stem and root barks of the plant *Erythrina abyssinica* were collected in the four districts of southwestern agro-ecological zone of Uganda. The Rubare subcounty in Ntungamo, the Rubindi subcounty in Mbarara, the Bugongi subcounty in Bushenyi and the Lugusulu subcounty in Rakai districts.

The collected *Erythrina abyssinica* materials were pressed and voucher specimen deposited with the Botany Department at Mbarara University of Science and Technology. The remaining plant materials were taken to Mbarara Zonal Agricultural Research and Development Institute (Mbarara ZARDI) for drying. The plants were dried in the shade at 25^0C for one

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week. The plant materials were then pounded into powder (in a mortar) for chemical extraction at the Uganda Natural Chemotherapeutics Research laboratories (UNCRL).

Figure 1. Picture of *Erythrina abyssinica*

Two hundred fifty grams (250gr) of freshly dried powdered root, stem barks and leaves were macerated in 2000 ml of 70% ethanol for 72 hours with intermittent shaking. Filtration through cotton wool was done to remove coarse particles (residues) and filter paper 12.5mm (Whitman®, No.1). The filtrate was concentrated on rota-vapour under reduced pressure at 40^0C. The concentrated extracts were later dried on weighed kidney dishes to a constant weight at 50^0C. The above procedures were repeated with water as solvent. The dried extracts were packed into universal bottles and kept at 4^0C until needed for bioassays.

c. Preparation of piperazine citrate stock concentration

A 100% *Piperazine citrate* powder was bought from a known Veterinary pharmacy in Kampala. Of this 30gr were weighed and dissolved in 600mls of Goodwin's solution to make a stock concentration of 50mg/ml of the drug as the highest concentrated dose level. The stock concentration was then serially diluted to make final concentrations of 25.00mg/ml, 12.50mg/ml and 6.25mg/ml for the experiment.

d. Experimental design

The conical flasks of 250ml capacity were labelled according to the different extracts and piperazine doses namely 0, 6.25, 12.5, 25 and 50 mg/ml. In each of the dose rate concentrations 200, 187.5, 175, 150 and 100 mls respectively of Goodwin solutions were added. The extracts added were 0, 12.5, 25, 50 and 100 mls respectively. The final volume per conical flask is 200mls of a solution containing Godwin solution and extracts. Ten worms were placed in each flask and these were incubated at 37˚C in water bath. The worms were monitored every 12hrs for a period of 48hrs (table 1).

The experiment had the following treatment groups: Negative control (N), Positive control involving three replicates of Piperazine citrate (P1,P2,P3), and the testing extracts, involving three replicates of Root bark (RB1, RB2, RB3), of Stem barks (SB1, SB2, SB3) and of Leaves (L1, L2,L3).

3. Worm motility assessment

In preliminary experiments, a criteria used for assessing the effects of crude plant extracts on the motility of adult *Ascaridia galli* was developed, combining the procedures previously described in literature [14], [28]. Worm motility was assessed at 12hours, 24hours, 36 hours and 48hours post treatment.

After 12, 24, 36 and 48 hours of incubation at 37^0C, the worms were gently removed from the testing treatment and re-suspended in Goodwin's physiological solution at the same temperature for 30 seconds for possible recovery of parasite motility. The worms were assessed for death or paralysis. A worm was considered to be motile if it moved in a sinusoidal motion when stimulated by water at 40-50°C. Similarly it was considered paralyzed if on stimulating it by water at 50^0C only part of the body responded either by raising the head and whether some parts showed autolysis and change of colour to pale white. Motility was also assessed by pressing the worm with an index finger, or using water at 50 - 60^0C to differentiate dead from paralyzed worms.

The percentage of immotile or dead worm was calculated as the number of dead worms divided by the total number of worms per flask multiplied by 100 to represent percentage paralyzed or dead.

4. Data analysis

The data collected were first entered in a laboratory counter book and then entered in a computer database (Microsoft Excel). The bioassay data was analyzed by the General Linear Model Procedures with multiple comparisons (Bonferroni method) and regression, using the Graph Pad Prism Version 5.01 software, Inc San Diego, CA USA. P value <0.05 was taken for significance level. The differences between the controls and treated means were analysed using one-way analysis of variance (ANOVA). Student t-test was used to separate

means where ANOVA showed significant difference. Graphs were drawn to illustrate the trends in activity by the *Erythrina* extracts and *Piperazine citrate* against *Ascaridia galli*.

The Comparison among specific plant extracts (Root-R, Stem-S and Leaves-L) and Districts (B-Bushenyi, M-Mbarara, N-Ntungamo, R-Rakai) was carried out using one way Anova then Post tested using Tukey test followed by Bonferroni post hoc *t*-test. P-value = 0.05 was used for significance level. The comparison in variations within concentrations of different extracts were conducted using Newman-Keuls Multiple Comparison Test P- value = 0.05 was used for significance level. The following parameters were tested: districts (Bushenyi, Mbarara, Ntungamo and Rakai), log transformation of the dose levels 0, 6.25, 12.5, 25 and 50 mg/ml, corresponding to 0.000, 0,796, 1.097, 1.398, 1.699.

5. Results presentation

The mean of action irrespective of the districts for Piperazine (P), Rootbark (R), Stem bark (S) and Leaves (L) at different concentrations 0, 6.25, 12.5, 25 and 50 mg/ml of the extracts. The *A. galli* were subjected to extracts for 48hours, monitored at each 12 hours interval, and results are detailed in Table 1.

Concentrations (mg/ml)	Total No. of worms used	Total No. of worms immobilized (paralysed +dead) after 48 hours			
		Piperazine	Root barks	Stem barks	Leaves
0	10	0	0	0	0
6.25	10	2.67±0.14	1.42±0.48	1.83±0.27	3.58±0.96
12.5	10	6.25±0.33	3.42±0.39	3.58±0.45	7.75±0.60
25	10	8.75±0.28	6.50±0.75	5.00±0.55	8.08±0.38
50	10	10.00	7.92±0.98	7.17±0.91	9.46±0.53

Table 1. Mean of action irrespective of the districts (Generally for Piperazine (P), Rootbark (R), Stem bark (S) and Leaves (L) for different concentrations of the extract

The rank correlation coefficient for the different extracts Piperazine (P), Root bark (R), Stem bark (S) and Leaves (L) at concentrations 0, 6.25,12.5,25,50 mg/ml are detailed in Figures 2 to 5. The *Piperazine citrate* has a rank correlation coefficient of R^2=0.7701; Root bark (R) R^2=0.8966, Stem bark (S) R^2=0.924 and Leaves R2=0.721

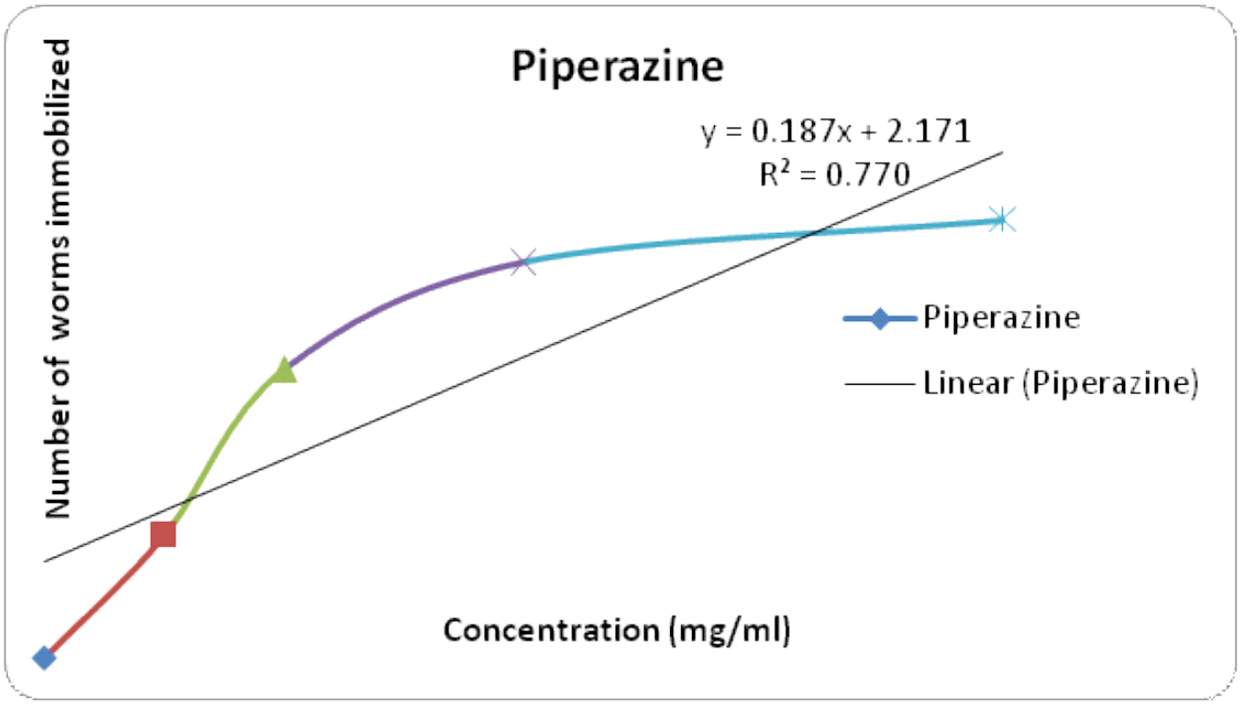

Figure 2. The effects of *Piperazine citrate* on the number of worms immobilized (paralysed +dead)

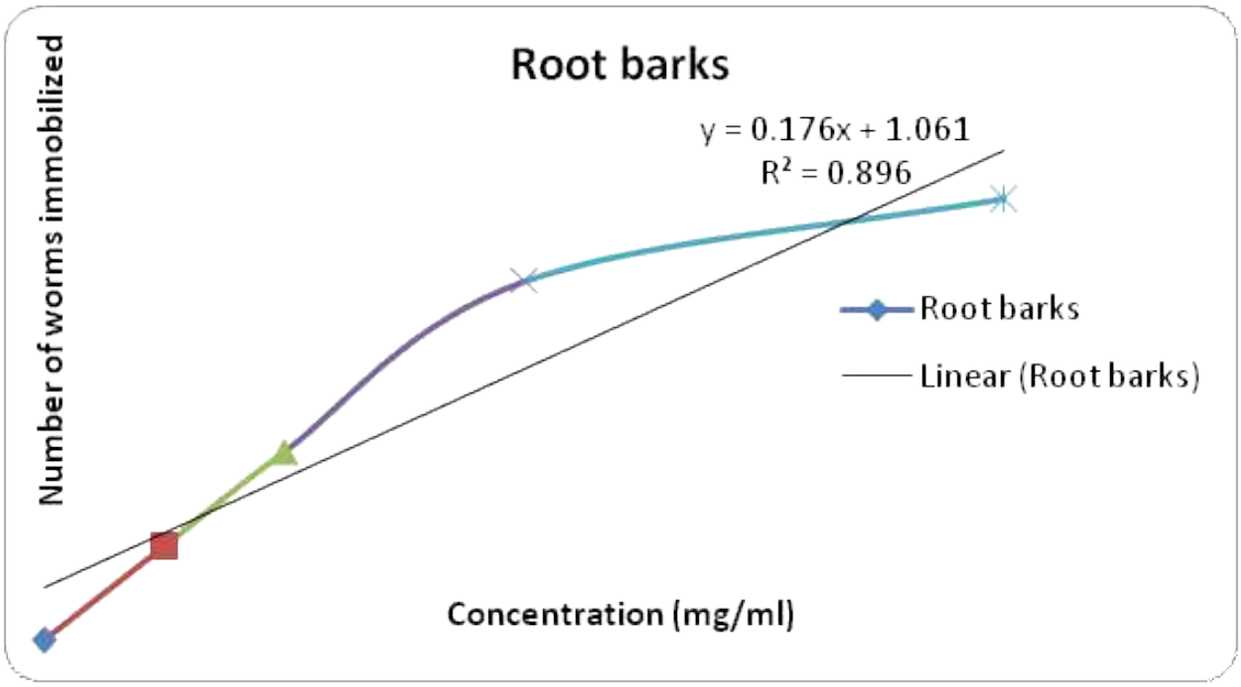

Figure 3. The effects of Root barks on the number of worms immobilized (paralysed +dead)

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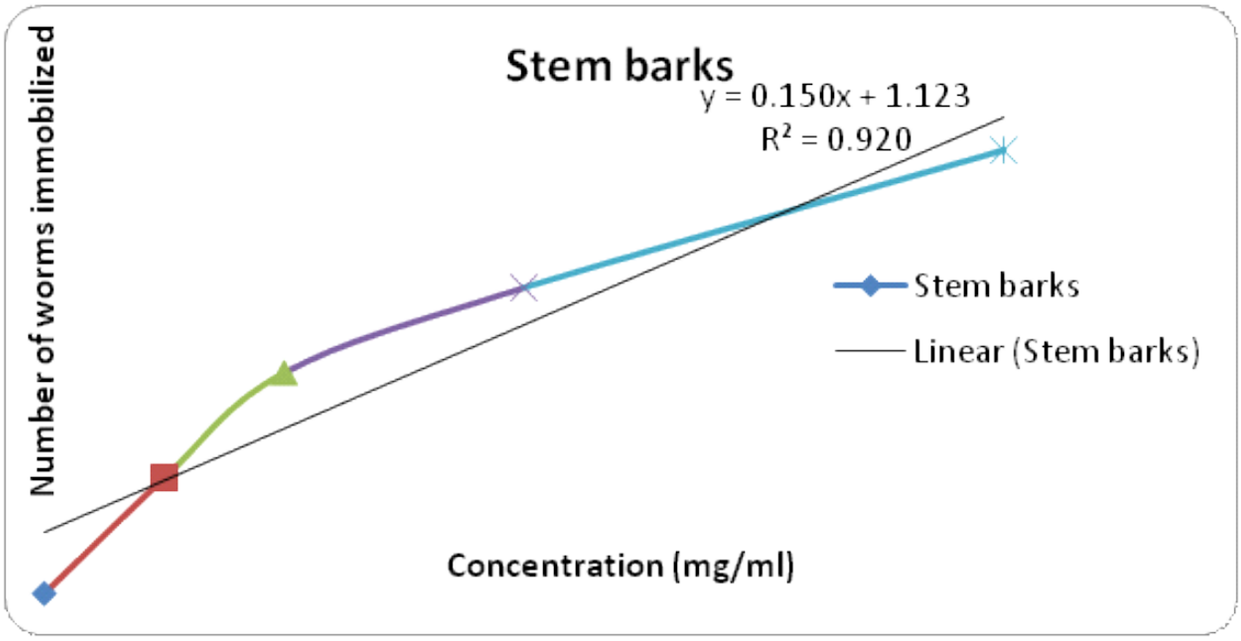

Figure 4. The effects of Stem barks on the number of worms immobilized (paralysed +dead)

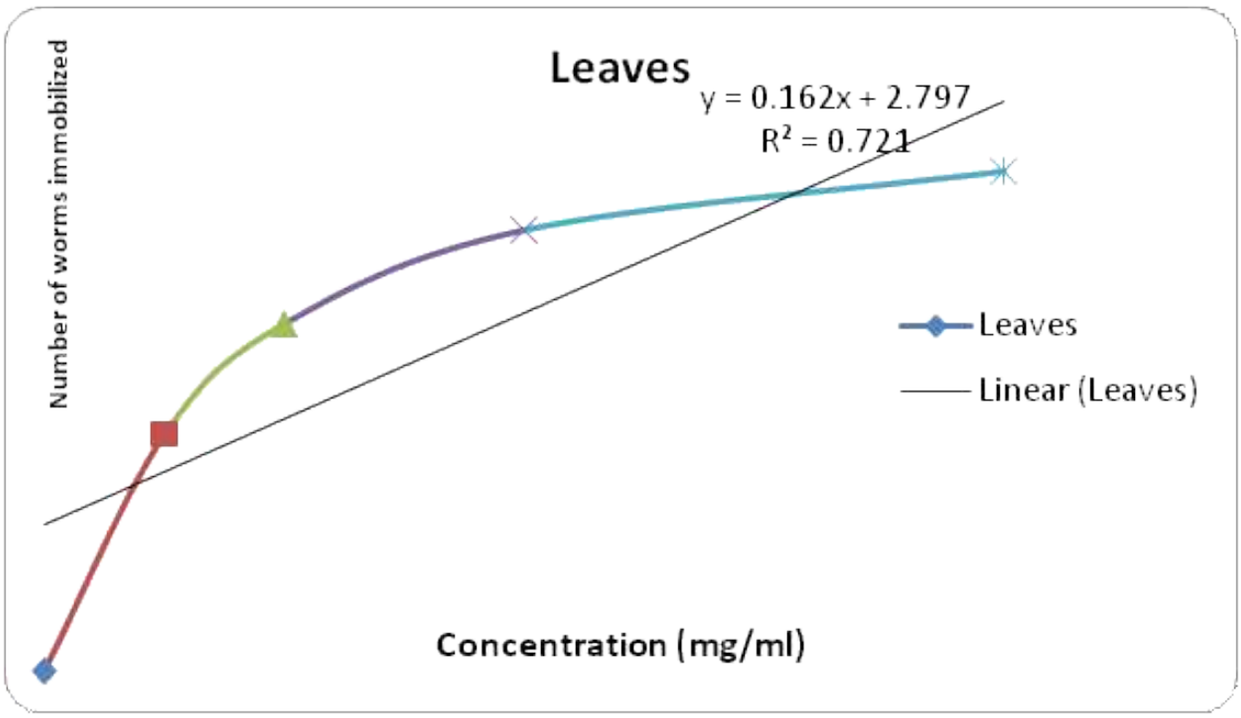

Figure 5. The effects of Leaves on the number of worms immobilized (paralysed +dead)

The study established the existence of a statistical significant relationship ($P<0.05$) between the Positive control (*Piperazine citrate*) at different concentrations and the extracts from different parts (root, stem and leaves) of the plant irrespective of the plant origin.

Further, the results found statistically insignificant differences ($p>0.05$) in activity against *A.galli* among specific plant extracts (Root-R, Stem-S and Leaves-L) and their location (Bushenyi, Mbarara, Ntungamo and Rakai)

The activity of the leaf extracts from Bushenyi, Mbarara, Ntungamo and Rakai districts were comparable to the conventional drug in the management of *Ascaridia galli as detailed* in Table 2.

Concentration mg/ml	0			0.79588			1.09691			1.39794			1.69897		
Plant parts	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L
Bushenyi	0	0	0	0	2	3	2	4	4	5	5	6	7	8	10
Mbarara	0	0	0	1	2	2	2	5	4	5	7	6	7	7	8
Ntungamo	0	0	0	1	2	3	2	4	5	5	5	7	7	8	9
Rakai	0	0	0	2	2	2	3	3	6	6	6	8	9	7	9

Table 2. Details the concentration of the extracts and the levels of worm immobilization (paralysed +dead) after 48 hours (R – Root barks; S – Stem barks; L – Leaves)

6. Discussion

The research showed that the leaves, stem barks and root barks of *Erythrina abyssinica* may be useful in poultry helminthosis control. This information supports its used as antihelminthic in ethno-veterinary medicine as previously defended [2-3]. The percentage of motility inhibition is an estimate of anthelmintic efficacy by comparing worm motility before and after incubation with plant extracts and *Piperazine citrate*. In this study the *Ascaridia galli* motility was assessed at 12, 24, 36 and 48 hours post treatment. The motility decreased with increasing extract concentration and increase in the incubation period. The anthelmintic property of plants is dependent on secondary plant metabolites [16-17] which in turn may depend on solvent of extraction [18] Paralyses of *Ascaridia galli* were very evident in treated groups that progressed till death of the parasite.

Piperazine is a GABA receptor agonist. Piperazine binds directly and selectively to muscle membrane GABA receptors, presumably causing hyperpolarization of nerve endings, resulting in flaccid paralysis of the worm. Similar observations have been reported showing that anthelmintic drugs kill worms either by starving them to death or by causing paralysis, which impairs the worm to store energy to meet their metabolic energy requirements [19]. The worms probably died from energy deficiencies (starvation) since they became paralyzed and fail to feed. It was further explained that interfering with feeding for up to 24 hours is sufficient to kill most adult parasites [12].

It is very crucial to note here that immobilization of the worms is ideal but it may lead to stenosis of the intestinal lumen. Thus it is important that a substance possess laxative prop-

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erties in order to remove the dead worm load, or it may induce the death of the host as a consequence of toxic syndromes.

It has been reported that some plant metabolites like tannins bind to glycoprotein on the cuticle of the parasite and disturb the physiological functions like motility [20].

The crude extracts yielded significant positive activity on *A.galli* as detailed in Table 1 within 48 hours. The study established that there were insignificant differences in plant parts in the various localities viz; Ntungamo, Mbarara, Bushenyi and Rakai. The differences between the therapeutic potential for root, stem and leaf vary between regions in a similar way/ proportion. The South Western Agro-ecological Zone of Uganda in which Ntungamo, Mbarara, Bushenyi and Rakai districts lies in the same agro ecological conditions, similar rainfall, temperature, humidity, soil ingredients and other biotic factors.

However, it has been found that ecological, genetic and environmental differences of plants harvested from the wild may vary in quality, consistency of active bio-compounds [21]. The variation in medicinal plants may also be linked to age of the plant, seasonal variation and geographical deviation at harvest site.

The study also indicated both plant extracts, in particular the leaves, and *Piperazine citrate* response did not differ significantly (Figures 2 to 5). The use the plant leaves crude extract as alternative de-wormer but dosages need to be standardized. This would make farmers save on cost of livestock production. These findings agree with previous farmer's claims that the plant is useful in the treatment of helminthosis [4,9,14].Repeated exposure to insufficient crude extract concentration could lead to worm resistance. This would explain the continued reports of helminthosis and low livestock productivity despite farmers use of medicinal plants to contain the parasites.

Ethanolic extraction was selected in this work to extract active substances from the root bark, stem bark and leaves of *Erythrina abyssinica*. Farmers use water to extract the active substances. There may be variation in extraction potential using aqueous and ethanol solvent systems due to the kind of bioactive substances extracted by the two solvents since different solvents extract different compounds depending on type of substances and polarity [22].

Further, alcohol is a "good for all-purpose" solvent for preliminary extraction [12,22]. In this study the use of ethanol has an added advantage of extract preservation and increasing the shelf life of the medicinal plant extracts. This would not only reduce labour of repeated preparation but also promote the plant species conservation.

The anthelmintic activities observed might be due the *Erythrina* condensed tannins, though synergy by other compounds could have enhanced the activity. The role of condensed tannins in helminth control has been demonstrated [23-24].

Chemically, tannins are polyphenolic compounds [30] and some synthetic phenolic anthelmintics, like niclosamide and oxyclozanide are said to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that tannins contained in the extracts of *Erythrina abyssinica* produced similar effects. It was also suggested

that tannins bind to free proteins in the gastrointestinal tract of host animal [2,25] or glycoprotein on the cuticle of the parasite disturbing the physiological functions like motility, feed absorption and reproduction [20,26-27,] or by interference with morphology and proteolytic activity of microbes [13,28] and cause death.

Alternatively, the presence of alkaloids salts which are physiologically active with sedative and analgesic properties could have contributed to the paralysis and consequent death of the worms. Alkaloids are toxic due to their stimulatory effects, leading to excitation of cells and neurological dysfunction.

7. Conclusion and recommendations

The study validates the farmers efforts, who have been using for long these medicinal plants and stem barks for management of diverse ailments in poultry and other livestock diseases. The findings of the current study provide evidence that *Erythrina abyssinica* can be used by local farmers to control poultry helminthosis. Our study found that leaves had very good activity on *Ascaridia galli* comparable with the conventional *piperazine citrate*. This finding provides a new innovation in the utilization of plants parts to solve helminthes problems in local chicken. The use of leaves is important for sustainable conservation of plants. Plants where the community use the root barks are more endangered than plants whereby the community use plant leaves. The use of leaves is an opportunity to conservation of *Erythrina abyssinica* without tampering with the root barks.

Nevertheless, there is the need to conduct acute toxicity test to establish safety of the plant extracts. The use *Erythrina abyssinica* leaves other than root or stem is sustainable way of conserving the medicinal plants.

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References

[1] Masimba E S, Mbiriri D T, Kashangura M T and Mutibvu T 2011: Indigenous practices for the control and treatment of ailments in Zimbabwe's village poultry. Livestock Research for Rural Development. Volume 23, Article #257. Retrieved September 4, 2012, from http://www.lrrd.org/lrrd23/12/masi23257.htm

[2] Kotze, A. C., Clifford, S., O'Grady, J., Behnke, J. M. and McCarthy, J. S., 2004. An in vitro larval motility assay to determine anthelmintic sensitivity for human hookworm and strongyloides species. American Journal of Tropical Medicine and Hygiene, 71, 5, 608-616.

[3] Mathias, E., D.V. Rangnekar, and C.M. McCorkle, 1999. Ethnoveterinary Medicine: Alternatives for Livestock Development. Proceedings of an International Conference held in Pune, India, on November 4-6, 1997. Volume 1: Selected Papers. BAIF Development Research Foundation, Pune, India.

[4] Nalule A S, Mbaria J M, Olila D and Kimenju J W , 2011: Ethnopharmacological practices in management of livestock helminthes by pastoral communities in the drylands of Uganda. Livestock Research for Rural Development. Volume 23, Article #36.Retrieved May 18, 2011, from http://www.lrrd.org/lrrd23/2/nalu23036.htm

[5] Athanasiadou S, Githiori J and Kyriazakis I, 2007 Medicinal plants for Helminth parasite control: facts and fictions. Animal (2007), 1:9, pp1397-1400.

[6] Gakuya D .W, 2001. Pharmacological and clinical evaluation of the anthelmintic activity of Albizia anthelmintica Brogn, Maerua edulis De wolf and Maerua subcordata De wolf plant extracts in sheep and mice. PhD thesis. University of Nairobi, Clinical Sciences Department; 2001.

[7] Githiori J B, 2004. Evaluation of anthelmintic properties of ethno veterinary plant preparations used as livestock dewormers by pastoralists and small holder farmers in Kenya. PhD thesis, Uppsala 2004. Acta Universitatis Agriculturae Sueciae Veterinaria 173.

[8] Hammond J A, Fieding D and Bishop S C, 1997 Prospects for plant anthelmintics in tropical veterinary medicine. Veterinary Research Communications 2: 213-228.

[9] Lagu C and Kayanja F I B ,2010: Medicinal plant extracts widely used in the control of Newcastle disease (NCD) and helminthosis among village chickens of South Western Uganda. Livestock Research for Rural Development. Volume 22, Article #200.Retrieved May 18, 2011, from http://www.lrrd.org/lrrd22/11/lagu22200.htm

[10] Anonymous (2011). Ascaridia galli infection. http://www.worldpoultry.net/diseases/ascaridia-galli-infection-d58.html retrieved on 30th June, 2011

[11] Lamson, P.D and Brown, H. W., 1936. Methods of testing the anthelmintic properties of ascaricides, American Journal of Hygiene, 23, 85-103.

[12] Nalule A S, Mbaria J M, Kimenju J W and Olila D 2012: Ascaricidal activity of Rhoicissus tridentata root-tuber ethanolic and water extracts. Livestock Research for Rural Development. Volume 24, Article #144. Retrieved September 4, 2012, from http://www.lrrd.org/lrrd24/8/nalu24144.htm.

[13] Waghorn, G.C., and McNabb, W.C., 2003. Consequences of plant phenolic compounds for productivity and health of ruminants. Proceedings of the Nutrition Society, 62, 383–392.

[14] Wasswa P and Olila D (2006). The in-vitro Ascaricidal activity of selected indigenous medicinal plants used in Ethno Veterinary practices in Uganda. African Journal of Traditional Complementary and alternative Medicines (2006) 3 (2).

[15] Donahue, M. J., Yacoub, N.J., Kaeini, M. R., Masaracchia, R. A., Harris, B .G., 1981. Glycogen metabolizing enzymes during starvation and feeding of A. suum maintained in a perfusion chamber. Journal of Parasitology, 67, 4, 505-510.

[16] Brookes, K.B and Katsoulis, L. C., 2006. Bioactive components of Rhoicissus tridentata: a pregnancy-related traditional medicine. South Africa Journal of Science, 102, 5-6, 267-272.

[17] Naido, V., 2005. Screening of four major plants commonly used in ethno veterinary medicine for antimicrobial and protozoal and antioxidant activity. Unpublished Master of Science thesis, University of Pretoria Ltd.

[18] Malu S. P., ObochI G. O., Edem C. A. and Nyong B. E. 2009. Effect of methods of extraction on phytochemical constituents and antibacterial properties of tetracarpidium conophorum seeds. Global journal of pure and applied sciences Vol 15, no. 3, 2009: 373-376

[19] Schoenian, S., 2008. Understanding anthelmintics (dewormers). Small Ruminant Info Series. Western Maryland Research & Education Center, University of Maryland Cooperative Extension.

[20] Thompson, D. P. and Geary, T. G., 1995. The structure and function of helminth surfaces. In: Marr J.J, (Edn). Biochemistry and Molecular Biology of Parasites. 1st Ed. New York: Academic Press 203–32.

[21] Street R.A., Stirk W.A and Staden Van (2008). South African Medicinal plant trade-Challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology 119 (2008) 705-710.

[22] Harborne, J. B. 1973. Phytochemical Methods. Chapman and Hall, London p. 113.

[23] Cenci F.B., Louvandini, H., McManus, C.M., Dell'Porto, A., Costa, D.M., Araújo, S.C., Minho, A.P., and Abdalla, A.L., 2007. Effects of condensed tannin from Acacia mearnsii on sheep infected naturally with gastrointestinal helminthes Veterinary Parasitology, 144, 1-2,132-137.

[24] Molan, A. L., Waghorn, G .C., Min, B .R. and McNabb, W. C., 2000.The effect of condensed tannins from seven herbages of Trichostrongylus columbriformis larval migration in vitro. Folia Parasitological, 47, 39-44.

[25] Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R. L., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: In vitro and in vivo studies. Veterinary Parasitology, 99, 205–19.

[26] Aerts, R.J., Barry, T.N., McNabb, W. C., 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agricultural Ecosystems Environment, 75, 1-12.

[27] Githiori, J.B., Athanasiadou, S. and Thamsborg, S. M. 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. Veterinary Parasitolology.

[28] Min, B. R., Barry, T. N., Attwood, G. T., and McNabb, W. C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Animal Feed Science and Technology, 106, 3–19.

[29] Nanyingi.M. O., Mbaria J.M, Lanyasunya.A.L., Wagate.C.G .,Koros.K.B., Kaburia.H.F., Munenge. R.W and Ogara W.O (2008). Ethnopharmacological survey of Samburu district, Kenya. Journal of Ethnobiology and Ethnomedicine 2008, 4:14

[30] Bate-Smith, E. C., 1962. The phenolic constituent of plants and their taxonomic significance, dicotyledons. Journal of the Linnean Society of London, Botany, 58, 95–103.

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