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In-Vitro Anthelmintic Bioactivity Study Of Eclipta prostrata L. (Whole Plant) Using Adult *Haemonchus contortus* worms A Case Study Of Migori County, Kenya

Sirama V[1], Kokwaro J[1], Owuor B[3], And Yusuf A,[2]
[1]*School of Biological Sciences, University of Nairobi, Box 30197-00100, Nairobi;*
[2]*Department of Chemistry, University of Nairobi, Box 30197-00100, Nairobi;*
[3]*Catholic University of Eastern Africa, Box 62157, Nairobi.*

Abstract: *The aim of this study was to investigate the anthelmintic activity of Eclipta prostrata (Asteraceae) which is used by traditional medicine practitioners in Migori County using adult Haemonchus contortus worm as a model.50gm of ground powder of Eclipta prostrata (whole plant) was extracted separately with 300 ml methanol, acetone and water. The yields of the extracts were 3.53 gm, 4.19 gm and 4.02 gm for methanol, acetone and water respectively. The anthelmintic activity of 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml concentrations of aqueous, acetone and methanol crude extracts of Eclipta prostrata (whole plant), were compared with the effect produced by the standard reference drug albendazole with Phosphate Buffered Saline (PBS) used as a negative control. Death of Haemonchus contortus worm was determined within a period of 24 hrs. Eclipta prostrata extract had mean mortality of 26.7-40.0 % at 6.25 mg/ml; 33.3-53.3 % at 12.5 mg/ml; 36.7-56.7 % at 25 mg/ml.All the extracts contained tannins and cardiac glycosides.*

Keywords: *Eclipta prostrata, Haemonchus contortus, In-vitro anthelmintic activity, Albendazole, Migori County, Kenya.*

I. Introduction

Disease has been an integral part of man and the search for remedies to combat it has been ongoing. In China, Japan and Korea, medicine has been extensively and safely used to alleviate various symptoms of diseases [1]. Worldwide, traditional herbal medicine has preoccupied mankind in his evolution [2]. The World Health Organization (WHO) estimates that 70-90 % of Africa's rural population relies on traditional medicine to meet its health needs, and thus recognizes herbal medicine as an essential component of primary health care (PHC) [2]. A large population of people in the developing countries depend on traditional medicine for PHC and it is important that these herbal remedies are investigated for their efficacy [3, 4].Studies of wild animals show that they instinctively eat certain plants to treat themselves from certain illnesses [5]. The whole plant or parts of it have been used to prepare drugs. Modes of preparation of these drugs include boiling, soaking, burning, pounding, chewing, heating and roasting [4].

Plants existing in different geographical regions exhibit varying concentrations of secondary metabolites [6]. Plant secondary metabolites offer the plants protection from animals, diseases, pests and adverse environmental conditions. Helminth infections (helminthiasis) are the most common infections in man that affect a large proportion of the world's population [7]. Most diseases caused by helminths are chronic and debilitating in nature. Helminthiasis is endemic in regions with poor sanitation and hygiene, malnutrition and crowded living condition. It has been estimated that about half of the world's population suffers from helminthiasis and the number is increasing. In the treatment of helminthiasis, anthelmintic drugs are used irrationally and recently anthelmintics use has been found to produce toxicity in human beings [7]. However the high costs of conventional anthelmintics has limited the effective control of the parasites. In some cases, wide spread use of low quality anthelmintics has enhanced development of resistance [7]. Hence the discovery of new plants containing bioactive substances that act as anthelmintics is considered a breakthrough in managing this disease [7].

Currently, pharmaceutical industries invest immense capital in seeking promising medicinal herbs with novel chemical compounds which could be used as disease therapies [8]. Several plants are used to manage these infections. Information on chemical composition of these plants can be generated for further advanced research work, which may include the isolation of these chemicals and their subsequent use in the development of more efficient and safe anthelmintic drugs. The research study focused on anthelmintic plants of Migori County providing essential information on their chemical composition and bioactivity.

1.1Traditional Medicine

This is the sum total of all the knowledge and practices, whether applicable or not, used in the diagnosis, prevention and elimination of physical, mental or social disequilibrium and relying exclusively on practical experience and observation handed down verbally or in writing from generation to generation [9, 4]. Traditional medicine utilizes plants/herbs, animal and mineral substances.

Many people in Kenya are already taking herbal medicines as self medication; these are usually prepared at home, or obtained from herbalists, pharmacies and supermarkets. In Kenya the registration of herbal medicines is done by the Pharmacy and Poisons Board (PPB); the national drug regulatory authority [2]. In Kenya medical doctors do not receive any training in herbal medicine while in Asia medical doctors acquire postgraduate degrees or diplomas in herbal medicine [2]. It is estimated that in Kenya, the doctor: patient ratio is 1:7142. However, complementary medicine practitioner: patient ratio is much better 1:987. Kenya faces several challenges in the integration of traditional herbal medicine in national health care. These include: lack of a national policy and regulatory framework; lack of healthy cooperation and communication between complementary medicine providers and medical practitioners; lack of a clear policy on Intellectual Property Rights (IPR) [2]. Since the 1960s forest cover in Kenya has greatly reduced from 12.5% to 2.5%. This is attributed to rapidly expanding population, illegal logging and acquisition of land for cultivation [10]. This has led to a major impact on medicinal plants, and loss of some species [11]. It is therefore important that plant biodiversity is conserved in order to protect our natural habitat in the long run [12]. This calls for systematic cultivation of medicinal plants in order to conserve biodiversity and protect endangered species [13].

In the recent years, the importance of herbal drugs in medicine has tremendously increased because of their association with fewer side effects [7]. Consequently, the demand for herbal formulations is increasing. The phytochemical constituents and their standardization have been accelerated with the development of analytical tools which makes this field important [7].

1.2 Anthelmintic Plants

These are plants that are used traditionally in expelling the worms that are parasitic in nature from the body by either stunning or killing them. They are also known as vermifuges or vermicides [4, 7].

1.3Helminth Parasites

Helminths are macroscopic worms causing a wide variety of diseases globally called helminthiases. There are three different kinds of helminthes: Platyhelminths (flat worms), Nemathelminths (non-segmented roundworms) and Annelida (segmented round worms). Those infecting human belong to the first two groups. Helminths are multi-cellular worms with sizes varying from 1mm to several metres in length. They develop through egg, larval (juvenile), and adult stages. Depending on the species, the infective stage is egg or larva [14].

In this study we used *Haemonchus contortus* adult worm (fig 1), found in the abomasums of sheep and goats [15]. It is a nematode belonging to the order Strongylida and is responsible for anaemia, bottle jaw (fluid accumulation in sub-mandibular tissues) and death of infected sheep and goats mainly during summer months in warm, humid climates [16]. It is cylindrically shaped, tapered at both ends, and has a complete digestive system [17]. It is a worldwide threat, but is more prevalent in sub-temperate and temperate regions under warm and wet conditions [17]. This parasite has a short life cycle of approximately three weeks. Grazing animals pick up the infective larvae on forages that are relatively short. Once in the rumen it continues to develop, move to the abomasum and become adults (20-30 mm). An adult female can lay thousands of eggs daily and can consume 200 microlitres of blood daily. An average of 10000 adults is enough to kill a sheep or a goat. The eggs (thin-shelled) are deposited in faeces, which then hatch to begin the life cycle again. During drought or very cold conditions a majority of larvae become dormant or die thus the transmission to the animal is very low [16].

Fig 1: *Haemonchus contortus* adult worm accessed from nematode.net

Although traditional medicine has been accepted worldwide, some of its aspects require attention if it is to be mainstreamed in health services. These include its imprecise nature of diagnosis, lack of precision in dosage, possible misuse of non-material aspects and practice of quackery [4]. Helminthiasis causes threat both to human and domestic animals world-wide. Study of anthelmintic medicinal plants could help in discovering alternative sources of medicine for the eradication of helminth parasites.

1.4 *Eclipta prostrata* (Asteraceae)

It is an erect or prostrate, small much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile, lanceolate, subentire, acute or sub-acute sparsely strigose with appressed hairs on both sides and with a tapering base, 2.5-7.5cm long, brown stem and small white flowers on a long stalk. It grows 3" tall [18, 1] (fig 2).

Fig 2: *Eclipta prostrata* (Asteraceae)

The herb has been known for its curative properties and has been utilized as antimytotoxic, analgesic, antibacterial, antiviral, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, anti-inflammatory, immunomodulatory, a good rejuvenator and as an anti-aging [18]. It is used as a tonic and diuretic in spleen enlargement, treatment of memory disorder, edema, rheumatic joint pains, fever and improving digestion [19]. It is also used in catarrhal jaundice and for skin diseases. The fresh juice of leaves has been used for jaundice, anemia, dysentery, eye diseases, asthma [1] increasing appetite, and as a mild bowel regulator [18].

The root has been reported to possess emetic and purgative property. The tincture of the plant is used for liver and kidney problem and it is also reported to have therapeutic potential against cardiovascular disorders. [18].A wide range of chemical compounds including coumestans which is the main active principle, alkaloids, thiophenes, flavonoids, polyacetylenes, triterpenes, carbohydrates, saponins, tannins, phenols and their glycosides have been isolated from this species [18, 20 and21].

The leaves contain stigmasterol, β-terthienylmethanol, wedelolactone, dimethylwedelolactone and demethylwedelolactone-7-glucoside. The roots give hentriacontanol and heptacosanol; the aerial part is reported to contain a phytosterol, β-amyrin in the n-hexane extract and luteolin-7-glucoside, β-glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone in polar solvent extract. The polypeptides isolated from the plant yield cystine, glutamic acid, phenyl alanine, tyrosine and methionine on hydrolysis. Nicotine and nicotinic acid are reported to occur in this plant [18]. There is limited availability and affordability of modern medicines thus most of the world's population depends to greater extent on traditional medical remedies [21]. Recently the use of anthelmintic drugs have been found to produce toxicity in human beings, so there is a need to develop safe anthelmintic drugs from plant sources [7]. There is a need to search for therapeutic new agents due to the increasing instances of drug resistance by helminthic agents of human and animal diseases.

The number of higher plant species on earth are estimated at 250,000-500,000, of these, only about 6% have been screened for biological activity and a reported 15% have been evaluated phytochemically [22] are a great number of plants with purported antiparasitic properties, which have not been reproduced under experimental conditions [23]. This study opens opportunity for new discoveries that would be beneficial in managing helminthic infections.

II. Materials And Methods

2.1. Area Of Study

Migori County is located in the western part of Kenya in Nyanza Province between latitude 0°.24' South and 0°.40' South and longitude 34° East and 34°.50' East. It covers an area of 2,597 km^2 and borders Kisii, Homabayand Narok counties (figure 3).According to 2009 census, Migori County has a population of approximately 917,170 of which 34% of the population lives in the urban areas. The proposed County capital is Migori which is a cosmopolitan town. The Luo ethnic group is demographically dominant. Other ethnic groups include the Kuria, Luhya and Kisii. Migori County has four district hospitals. There are also clinics and dispensaries distributed within the County.

Migori County is divided into thirteen divisions namely Karungu, Nyatike, Muhuru, Suba East, Suba West, Uriri, Awendo, Rongo, Mabera, Masaba, Kehancha, Kegonga and Ntimaru (figure 4).Migori County experiences high temperatures of 21 degrees Celsius during the cold season and 35 degrees Celsius during the hot season. Rainfall is received in two seasons (March-May; October-December) with an annual average of 1200 mm. The County has an altitude of 1000metres.The major economic activity undertaken by most of the residents of Migori County is agriculture with the main commercial crops being sugarcane and tobacco. Other economic activities include fishing, mining and entrepreneurship.

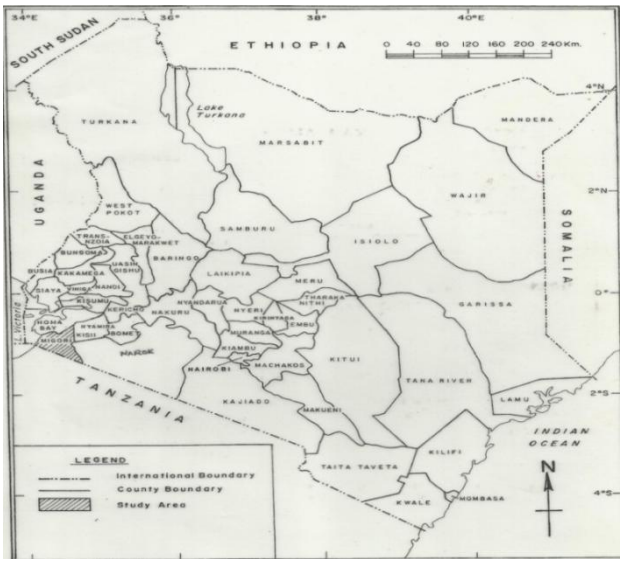

Fig 3: Map of Kenya showing the location of Migori County

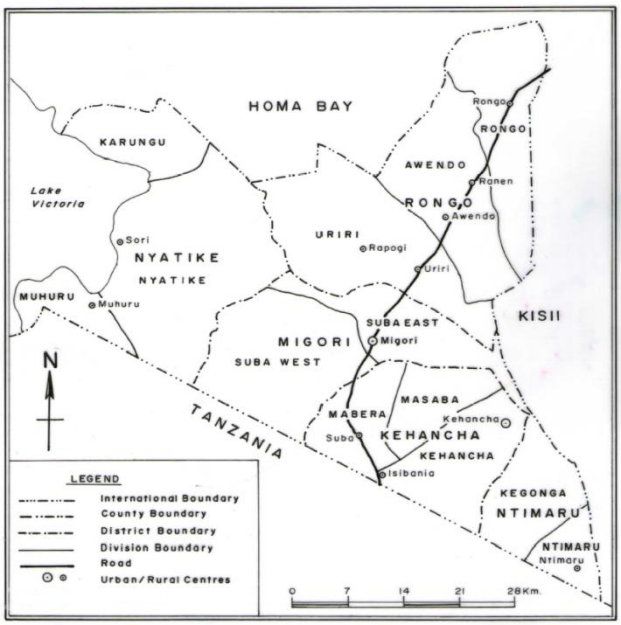

Fig 4: Map of Migori County showing thirteen Divisions

2.2 Collection Of Ethnobotanical Data

A field survey was done prior to data collection, during which, a list of herbalists was prepared with the assistance of rural dwellers and the local authorities (chiefs, Assistant chiefs) of MigoriCounty. Thereafter, information on the anthelmintic plants was collected for two months (August 2013 and September 2013). During this period, identified herbalists were visited in their homes and interviewed on their knowledge of anthelmintic plants. As such, the sampling was intentionally non-random under the assumption that herbalists would provide more specific and higher quality information concerning anthelmintic plants [24].

Ethnobotanical data was collected in all the thirteen Divisions in the County. Data collection was based on open ended interviews of the herbalists (medical practitioners). A questionnaire was used and for any additional information, complementary questions were asked [25]. Twenty six (26) herbalists between the ages 20-69 years (10 men and 16 women) were interviewed on plants used as anthelmintics. For every plant cited, vernacular name, parts used, mode of preparation and administration was recorded. Guided tours to observe and collect the plants mentioned for identification and laboratory studies were done with the help of respondents. Ethnobotanical data was compiled from field notes, herbarium sheets and available literature. Specimenof *E. prostrata* was collected in duplicate; one specimen was used for preliminary field identification with the help of Floras [26, 27] while the other was pressed and transported to the University of Nairobi herbarium (NAI) for authentic identification by comparing with the permanently prepared herbarium collections.

2.3 Selection Of Priority Plant

Priority plant was selected based on a survey carried out between August 2013 and September 2013 in Migori County. The frequency report as an anthelmintic agent by the respondents was prepared. *Eclipta prostrata* which had the highest frequency was selected and the whole plant used for bioactivity tests.

2.4 Collection Of *Haemonchus contortus* Worms

H. contortus worms were collected from the abomasums of freshly slaughtered sheep at Burma abattoir in Nairobi. The worms were washed with distilled water (1 litre) then suspended in 500 ml of phosphate buffer saline (PBS) (prepared by dissolving 0.85g of sodium chloride and 1g glucose in 1 litre of distilled water) [28].

2.5 Preparation Of The Plant Extract

Eclipta prostrata (whole plant) was washed with water, dried and then chopped into small pieces; this was then dried under a shade for three weeks and then ground into a powder using an electric mill [29]. It was then packed in a labeled packet. 50 g of this powder was soaked separately in 300 ml of methanol, 300 ml of acetone, and 300 ml of water in 500 ml conical flasks, covered with aluminium foil for 72 hrs and then filtered using the Whatman filter paper. The methanol and acetone extracts were each evaporated on a rotary evaporator at 60°C to obtain crude extracts which were transferred to separate marked vials which were then placed in an oven at 40°C for 2 hrs to dry the plant extracts into powder. Methanol and acetone extracts gave 3.53 grams and 4.19 grams respectively. 4.02 grams of water extract was realized. Water extract was deep frozen, freeze dried into powder then placed in a separate marked vial. The sample vials were kept at 4ºC for further use [28].

2.51 Test For Tannins

The dried powdered extract samples were weighed into 0.5 mgs, boiled in 10 ml of distilled water in test tubes and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration [30].

2.5.2 Test For Saponins

The dried powdered extract samples were weighed into 0.5 mgs, added to 5 ml of distilled water in test tubes and shaken vigorously for a stable persistent froth to occur. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion [30].

2.5.3 Test For Cardiac Glycosides (Keller-Killani Test)

The dried powdered extract samples were weight into 0.5 mgs, boiled in 10 ml of distilled water in test tubes then 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of 0.1% ferric chloride solution. 1 ml of concentrated sulphuric acid was then added. A brown ring at the interface indicated the presence of cardiac glycosides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer [28].

2.5.4In-Vitro Anthelmintic Activity

This was carried out as described by [28] with minor modification in the extract concentrations used. 0.625 gm, 1.25 gm and 2.5 gm of each powdered extract was dissolved in 5 ml of dimethylsulfoxide (DMSO) and made to 100 ml mark using distilled water to make 6.25 mg/ml, 12.5 mg/ml and 25mg/ml solutions [31]. Filter paper discs, 6 mm in diameter each impregnated with the above extract solutions were dried at room temperature to evaporate the DMSO. Ten (10) adult *Haemonchus contortus* worms were placed into a sterile Petri dish containing 10 ml of phosphate buffered saline (PBS). The filter paper discs was added and agitated; the same was done with the other filter discs impregnated with the other solvent extracts.

After 24 hours, the worms were removed from the Petri dish and then suspended in PBS for 30 minutes for possible recovery of their motility. Death was concluded when the worm lost their motility coupled with fading away of their body colour [32]. The number of motile (alive) and immotile (dead) worms were counted using a hand lens and recorded. The above procedure was repeated for all the other plants extracts. Albendazole (0.55mg/ml) was used as a reference drug (positive control) while PBS was used as a negative control. Worm motility and mortality was used as the rationale for anthelmintic activity.

2.6 Statistical Analysis

Descriptive statistics were used to analyse the data whereby applications in MS Excel® 2007 spread sheet were utilized to make simple calculations. The results obtained for anthelmintic activity were given as mean value ± standard deviation and the data were subjected to statistical analysis using analysis of variance (ANOVA) to determine whether there were significant differences in activity of the plant extracts at different concentrations used.

III. Results And Discussion

3.1 Ethnobotany Of The Identified Anthelmintic Plants

The study identified twenty one (21) anthelmintic plants distributed among thirteen (13) families and 21 genera. The plant's botanical, local name, description and their mode of preparation are given in table 1. The frequency of usage of the plants by the herbalists was used to pick *Eclipta prostrata* for bioassay.

Table 1: Anthelmintic plant identified during the study.

Botanical name	Vernacular name	Family	Habit	Parts used	Modeof preparation	Number of Independent Reports (IR)	Ranking
Bidens pilosa VOO 017/2013	Anyiego	Asteraceae	Herb	Whole	Decoction	7	16
Tamarindus indica VOO 014/2013	Chwaa	Leguminosaesubfam. Ceasalpinioideae	Tree	Bark	Concoction	15	10
Combretum collinum VOO 015/2013	Keyo	Combretaceae	Tree	Roots	Decoction	6	17
Solanecio mannii VOO 004/2013	Maroo	Asteraceae	Shrub	Leaves	Infusion	21	5
Leonotis nepetifolia VOO 005/2013	Nyanyodhi	Lamiaceae	Herb	Leaves	Decoction	5	18
Sclerocarya birrea VOO 010/2013	Ng'ong'o	Anacardiaceae	Tree	Bark	Decoction	11	13
Albizia coriaria VOO 006/2013	Ober	Leguminosaesubfam. Mimosoideae	Tree	Leaves	Infusion	20	6
Euclea divinorum VOO 012/2013	Ochol	Ebenaceae	Tree	Roots	Decoction	8	15
Aloe secundiflora VOO 019/2013	Ogaka	Aloaceae	Herb	Leaves, roots	Decoction	17	8
Plectranthus barbatus VOO 011/2013	Okita	Lamiaceae	Shrub	Leaves	Decoction	24	3
Rotheca myricoides VOO OO2/2013	Okwero	Verbenaceae	Herb	Roots	Infusion	16	9
Ximenia americana VOO 008/2013	Olemo	Olacaceae	Tree	Roots	Decoction	12	12
Vernonia amygdalina VOO 003/2013	Oluswa	Asteraceae	Tree	Leaves, roots	Infusion	25	2
Hypitis suaveolens VOO 021/2013	Oluwondara	Lamiaceae	Herb	Whole	Decoction	1	21
Erythrina abyssinica VOO 009/2013	Orembe	Leguminosaesubfam. Papilionoideae	Tree	Bark	Decoction	10	14
Eclipta prostrata VOO 020/2013	Osieko	Asteraceae	Herb	Whole plant	Infusion	26	1
Cucumis aculeatus	Otangle	Cucurbitaceae	Herb	Fruits	Decoction	23	4

VOO 018/2013							
Harrisonia abyssinica VOO 013/2013	Pedo	Simaroubaceae	Tree	Roots	Infusion	4	19
Carica papaya VOO 007/2013	Poipoi	Caricaceae	Tree	Roots	Decoction	18	7
Searsia natalensis VOO 016/2013	Sangla	Anacardiaceae	Tree	Roots	Decoction	2	20
Kigelia africana VOO 001/2013	Yago	Bignoniaceae	Tree	Bark	Concoction	14	11

50 grams of powdered plant was soaked separately in acetone, methanol and distilled water to extract the plant compounds. Each of the crude plant extract obtained was weighed to determine their yield. Percentage yield was then calculated as follows:

Percentage yield=Quantity of Extract /Quantity of plant material X 100

The results are given in table 2.

Table 2: mass and percentage yield of crude plant extracts

Plant species	**Methanol extract**		**Acetone extract**		**Water extract**		
	Yield (grams)	**Percentage yield (%)**	**Yield (grams)**	**Percentage yield (%)**	**Yield (grams)**	**Percentage yield (%)**	**Average yield (grams)**
E. prostrata (whole)	3.53	7.06	4.19	8.38	4.02	8.04	3.91

The yields of solvent extracts in decreasing order were acetone, water and methanol.

3.2 Phytochemical Analysis Of Crude Plant Extracts For Secondary Metabolites

Extracts of *Eclipta prostrata* was screened for tannins, saponins and cardiac glycosides using standard procedures [28]. The results were as given in table 3

Table 3: Phytochemical screening for each crude extracts for secondary metabolites.

Solvent / Secondary metabolites screened / Plant species	Methanol			Acetone			Distilled water		
	Tannins	Saponins	Cardiac glycosides	Tannins	Saponins	Cardiac glycosides	Tannins	Saponins	Cardiac glycosides
E. prostrata (whole)	+	-	+	+	-	+	+	-	+

Key: + = Present, - = Absent

The secondary metabolites present were tannins and cardiac glycosides.

3.3 *In-Vitro* Anthelmintic Activity Of Crude Plant Extracts

Each of the solvent crude plant extract at concentrations of 6.25 mg/ml, 12.5 mg/ml and 25mg/ml was tested in triplicate for anthelmintic potential. Mean mortality at various concentrations were calculated as represented in table 4.

Table 4: Mean mortality ± SD of the extract concentrations used.

Plant species	**Extract**	**Mean mortality ± SD**		
		6.25 mg/ml	**12.5 mg/ml**	**25 mg/ml**
Eclipta prostrata (whole)	Acetone	2.67±0.577	3.33±0.577	3.67±0.577
	Methanol	4.00±1.000	5.33±0.577	7.67±0.577
	Aqueous	3.00±0.000	3.67±0.577	5.33±0.577
Albendazole	0.55mg/ml	10.00±0.000	10.00±0.000	10.00±0.000
PBS	10 ml	0.00±0.000	0.00±0.000	0.00±0.000

Eclipta prostrata whole plant extract had mean mortality of 26.7-40.0 % at 6.25 mg/ml; 33.3-53.3 % at 12.5 mg/ml and 36.7-56.7% at 25 mg/ml. The activity in decreasing order of the extracts was methanol, water and acetone. There was significant difference in worm mortality of the solvent extracts at the various concentrations (6.25, 12.5, 25 mg/ml) used.

IV. Conclusions

E. prostrata (whole plant) extract had tannins and cardiac glycosides. Methanolic extract of the plant used in bioactivity studies gave the most activity followed by water; acetone gave the least potent extract. In all cases activity increased with concentration of the extract. *Eclipta prostrata,* can therefore be used in seeking new or alternative medicine for the treatment of helminth parasites.

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