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In-vitro anthelmintic activity study of *Plectranthus barbatus* Andr. Leaves on adult *haemonchus contortus* worms

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Abstract

The study investigated anthelmintic activity of *Plectranthus barbatus* (leaves) used by traditional medicine practitioners of Migori County using adult *Haemonchus contortus* worm as a model. 50 gm of ground powder of *Plectranthus barbatus* (leaves) was extracted separately with 300 ml each of methanol, acetone and water. The yields of the extracts were 3.32 gm, 3.98 gm and 3.36 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 *Plectranthus barbatus* (leaves), 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. *Plectranthus barbatus* (leaves) extract had mean mortality of 0-16.7 % at 6.25 mg/ml; 3.3-26.7 % at 12.5 mg/ml; 6.7-33.3 % at 25 mg/ml. All the extracts contained tannins only.

Keywords: *Plectranthus barbatus*, *Haemonchus contortus*, *In-vitro* anthelmintic activity, Albendazole, Migori County.

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1. Introduction

The search for remedies to combat disease has over the years engaged the minds of scientists. In Asian countries like China, Japan and Korea, medicine has been extensively used safely and effectively to alleviate disease [1]. 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 [2]. It is important therefore for herbal remedies to be investigated for their efficacy by determining their chemical composition and safety [3,4]. Studies of wild animals show that they instinctively eat certain plants to treat themselves from certain illnesses [5]. Humans have at times used the whole plant or parts of it to prepare drugs using methods like boiling, soaking, burning, pounding, chewing, heating or roasting [4].

Much of the traditional medicine research has centered on the medicinal value and efficacy of herbs and other pharmacopoeia [6].

Helminth infections (helminthiasis) are the most common infections in man that affect large proportions 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, hygiene, malnutrition and crowded living conditions. It has been estimated that about half of the world's population suffers from helminthiasis. In the treatment of helminthiasis, anthelmintic drugs have been found to produce toxicity in human beings [7]. However, the high costs of conventional anthelmintics have limited effective control of the parasites. In some cases, wide spread use of conventional 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]. Helminths pose a large threat to public health and contribute to the prevalence of anaemia, eosinophilia, pneumonia and malnutrition [8]. Because of the prevalence and impact of these parasitic worms, anthelmintic drug discovery is a priority in the pharmaceutical industry [7]. This research study focused on the possible use of *Plectranthus barbatus,* as an anthelmintic plant.

2. Materials and Methods

2.1 Area of Study

Migori County is located in the western part of Kenya in Nyanza Province between latitude 0º.24' South and 0º.40' South and longitude 34º East and 34º.50' East. It covers an area of 2,597 km2 and borders Kisii, Homabay and Narok counties (see 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. The Luo ethnic group is demographically dominant. Other ethnic groups include the Kuria, Luhya and Kisii. 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 100 metres.

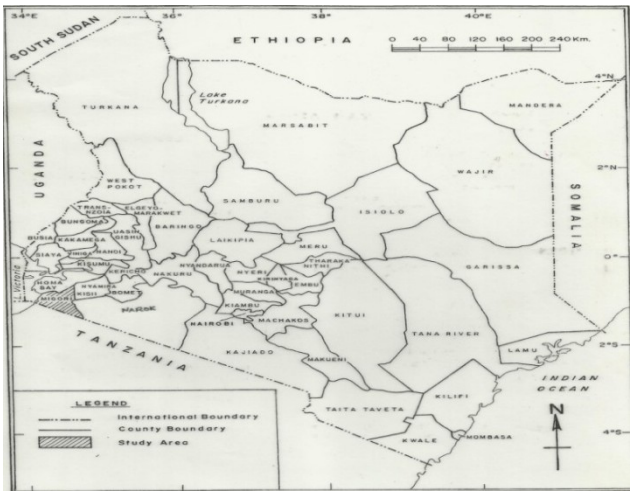

Fig 2: Map of Kenya showing the location of Migori County

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 Migori County. 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 [12].

Ethnobotanical data was collected in all the thirteen Divisions in the County (fig 4). 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 [13]. 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.

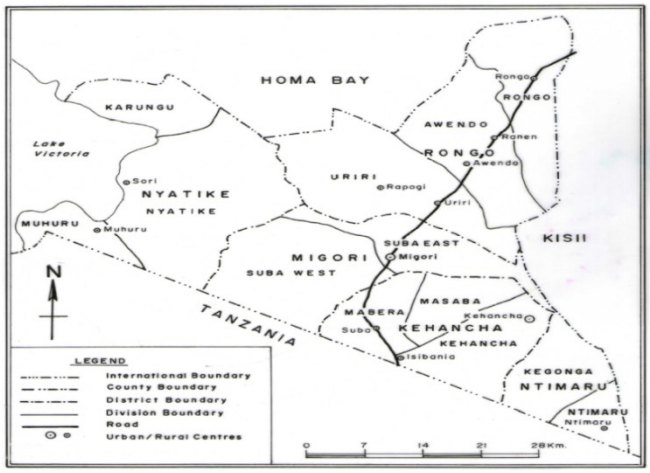

Fig 3: Map of Migori County showing thirteen Divisions

Plant specimens were collected in duplicate; one specimen was used for preliminary identification in the field with the help of floras [14, 15] 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 at the NAI herbarium.

2.3 Selection of Priority Plants

Priority plants were 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. *P. barbatus* was selected and the leaves 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) which was prepared by dissolving 0.85g of sodium chloride and 1g glucose in 1 litre of distilled water. They were then transported to the Zoology laboratory at School of Biological Sciences, Chiromo campus, University of Nairobi in an air tight can where authentication was done. They were then left for 2 hrs to acclimatize before beginning tests [16].

2.5 Preparation of the Plant Extracts

Plectranthus barbatus (leaves) 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 [17]. 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 3.32 grams and 3.98 grams respectively. 3.36 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 [16].

2.5.1 Test for tannins:

0.5 mg of each of the dried powdered extract sample was boiled in 10 ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration [18].

2.5.2 Test for saponins:

0.5 mg of each of the dried powdered extract sample was added to 5 ml of distilled water 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 [18].

2.5.3 Test for cardiac glycosides (Keller-Killani test):

0.5 mg of each of the dried powdered extract sample was boiled in 10 ml of distilled water then 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of 0.1% ferric chloride solution. This was then underlayed with 1 ml of concentrated sulphuric acid. 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 [16].

2.6 *In-vitro* Anthelmintic Activity

This was carried out as described by [16] 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 [19]. 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 disc containing the extract 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 [20]. The number of motile (alive) and immotile (dead) worms were counted using a hand lens and recorded. Triplicates were performed for each treatment. Albendazole (0.55mg/ml) was used as a reference drug (positive control). PBS was used as a negative control.

2.7 Statistical Methods

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.

3.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 plants botanical, local names, description and their mode of preparation are given in table 1

Table 1: Anthelmintic plants identified during the study

Botanical name	Vernacular name	Family	Habit	Parts used	Mode of 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	Leguminosae subfam. 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	Leguminosae subfam. Mimosoideae	Tree	Leaves	Infusion	20	6
Euclea divinorum VOO 012/2013	Ochol	Ebenaceae	Tree	Roots	Decoction	8	15
Aloesecundiflora 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	Oluwo ndara	Lamiaceae	Herb	Whole	Decoction	1	21
Erythrina abyssinica VOO 009/2013	Orembe	Leguminosae subfam. Papilionoideae	Tree	Bark	Decoction	10	14
Eclipta prostrata VOO 020/2013	Osieko	Asteraceae	Herb	Whole plant	Infusion	26	1
Cucumis aculeatus VOO 018/2013	Otangle	Cucurbitaceae	Herb	Fruits	Decoction	23	4
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

3.2 *Plectranthus barbatus* Andr. (Lamiaceae) – Okita

Fig 4: *Plectranthus barbatus* (Lamiaceae)

A shrub up to 4 m high. Leaves up to 10 cm long, elliptic or ovate, base attenuate to truncate, apex obtuse to acute, edge crenate, softly hairy, velvety. Flowers bright purple-blue, up to 2.5 cm long [15]. Leaf decoction is taken orally. Usually suitable for patients who have stomach ache.

Fifty (50) grams of each powdered plant extract 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: Yield 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)
P. barbatus (leaves)	3.32	6.64	3.98	7.96	3.36	6.72	3.55

Acetone gave the highest yield (3.98 gm), followed by water (3.36 gm) and lastly methanol (3.32 gm).

3.4 Phytochemical Analysis of Crude Plant Extracts for Secondary Metabolites

Extracts of each priority species was screened for tannins, saponins and cardiac glycosides using standard procedures [16]. The results are given in table 3.

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

Solvent	Methanol			Acetone			Distilled water		
Secondary metabolites screened	Tannins	Saponins	Cardiac glycosides	Tannins	Saponins	Cardiac glycosides	Tannins	Saponins	Cardiac glycosides
(Plant Species) *P. barbatus* (leaves)	+	-	-	+	-	-	+	-	-

Key: + = Present, - = Absent

P. barbatus had tannins only in all the extracts.

3.4 *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
Plectranthus barbatus (leaves)	Acetone	0.00±0.000	0.33±0.577	0.67±0.577
	Methanol	1.67±0.577	2.33±0.577	3.33±0.577
	Aqueous	0.33±0.577	0.67±0.577	1.33±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

Mortality increases in the order methanol, water and acetone for all concentrations. *Plectranthus barbatus* leaf extract had mean mortality of 0-16.7 % at 6.25 mg/ml; 3.3-26.7 % at 12.5 mg/ml; 6.7-33.3 % at 25 mg/ml. There was significant difference in worm mortality of the solvent extracts at the various concentrations (6.25, 12.5, 25 mg/ml) used.

The bioactivity study indicates that *Plectranthus barbutus* is a potential antihelmintic. This research indicates possible development of conventional Anthelmintic drugs using biochemical extracts from the plant.

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