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Tithonia diversifolia and Senna didymobotrya Extracts Show Effectiveness Against Fleas Without Harming Mammals

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Abstract

Traditional uses for the bio-pesticides Tithonia diversifolia and Senna didymobotrya are described here. There is a lack of evidence supporting their utility in flea control, and there is also concern about the safety of their aqueous extract.

Methods Acute toxicity in Wister rats and cutaneous and ocular irritation in Newzealand albino rabbit were evaluated using a technique previously published, and the antifleas activity of Tithonia diversifolia and Senna didymobotrya were compared with Chrysanthemum cinerariifolium. We started by making crude aqueous extracts of the flowers and leaves of T. diversifolia, S. didymobotrya, and C. cinerariifolium, and then we diluted those extracts and the placebos in a series of dilutions. Plant extracts were applied to strips of Whatman's filter paper no. 1 and tested for their antifleas properties using fleas collected from stray dogs. After 24 and 48 hours, we counted the number of alive fleas in the polypylene tubes to assess the level of activity.
The most effective treatment against fleas was found in T. diversifolia (93%), followed by C. cinerariifolium (90%) and S. didymobotrya (66.3%). The LD50 for all three plant extracts evaluated was more than 2000 mg/kg, and there were no symptoms of ocular or skin toxicity.
In conclusion, further research is needed to determine whether the flowers of T. diversifolia can be utilized to manage jigger flea populations.

Key words: T diversifolia flowers; Fleas control; Jiggers

1. Introduction

Fleas are a kind of ectoparasite that may jump from a human host to a domestic animal and then back to the human again. Dogs in colder climates are not immune to flea infestation since the fleas' capacity to breed exclusively in homes makes them present all year long. Flea bites may result in pruritus and flea allergic dermatitis (FAD), but they can have other, less serious, pathogenic consequences. Controlling the flea population is essential for preventing FAD; this requires the consistent use of chemical agents, often in the form of topical preparations but in rare cases orally [1].

Fleas are not only annoying, but they may also spread illness. There are three human diseases that are linked to fleas. Plagues include bubonic, pneumonic, and septicemic. Murine typhus fever is caused by the bacterium rickettsia typhi, and it is spread mostly by rat and cat fleas. Humans get the disease when their bodies come into contact with infected fleas' dried feces and crushed corpses. The female sand fleas, chigoes, or jigger fleas may cause irritation by burrowing into the skin. Jigger infestations may range from just a few to hundreds of parasitic worms per host. The whole process, from egg to adult, may take as little as 18 days under ideal circumstances [2].

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The make-up of bacterial communities is governed by arthropods like fleas and ticks [3]. Several different types of vector-borne illnesses are transmitted by fleas [4]. Plague is caused in humans by the bacterium Yersinia pestis. Only a small percentage of fleas actually encounter people on a regular basis, and none of the flea species are unique to humans. However, in plague-endemic parts of Africa [5, 6], fleas associated with humans (Pulex irritans, Ctenocephalides felis, and X.cheopis) have been reported in high numbers in human houses. Cat fleas (C fellis) are implicated in plague outbreaks, such as the one in Northwest Uganda [4], while human fleas (P irritans) are linked to human-to-human transmission of the disease. Bubonic plague, the most frequent form, septic plague (plague without bubo), pneumonic plague, meningitis, and pharyngitis are among clinical symptoms of the plague [6]. If left untreated, pneumonic plague may be lethal within hours. Parasitizing domesticated animals may cause significant economic losses and spread illness, thus controlling fleas and ticks is important [7]. Fleas, ticks, mites, and body lice are all vectors for Rickettsia typhi. An arthropod transmits the gram-negative, obligatory intracellular bacteria that originated in rats to humans [8].

According to Bitam et al. [4], Fleas of the genus Tunga may transmit a wide variety of bacteria to humans, including Clostridium tetani, Streptococcus pyogens, Satulococcus aureus, Klebsiella aerogenes, Enterobacter agglomerans, Escherichia coli, and other enterobacteriaceae, which cause secondary bacterial infections in the lesions. Six thousand two hundred Kenyan schoolchildren were found to have Tungiasis in 2014, according to a research conducted in the Kandara Sub-Counties of Murang'a County [9]. Most people with jiggers utilize tools like needles and tweezers to get rid of them [10]. Sesquiterpene lactones and diterpenoids are present in Tithonia species, and they have biological effects against insects [11]. Tithonia diversifolia is utilized for jigger flea management and control in the Ikolomani Division of Kakamega County, Kenya [12]. This is because

There is little data on the efficacy of crude plant extracts against animal-hosting fleas, such as Tithonia diversifolia and Senna didymobotrya. Furthermore, there has not been enough information given on the toxicity and safety profiles of these plants in diverse species. Since pesticides have been linked to unintentional poisoning, it is crucial to study the LD50 of plant extracts from these plants. If the extracts from the two plants are found to be effective against fleas, then further research should be conducted to see if the same extracts can be effective against the sand flea, a jigger-causing flea that is said to be a menace to the poor residents of Murang'a County and other regions of Kenya.

The purpose of this research was to examine the in vitro antiflea efficacy of crude extracts of Tithonia diversifolia flowers and leaves and Senna didymobotrya leaves. The acute toxicity and sensitization of the skin to these extracts, as well as their various purposes, were also investigated.

2. Materials and Methods

Approvals of the study

The study was approved by National Commission for Science Technology and Innovations (NACOSTI) and also Biosafety, Animal Use and Ethics Committee (BAUEC) of University of Nairobi.

Experimental animals

The acute dermal irritation/corrosion (OECD, 404) and ocular irritation test (OECD, 405) were conducted on young adult female nulliparous and non-pregnant Newzealand albino rabbits procured from the University of Nairobi animal house. Acute toxicity testing of the extracts were conducted on adult (8-12 week old), female, nulliparous and non-pregnant Wister rats weighing 90-130 g that were procured from the University of Nairobi animal house. All animals in these trials were kept singly and given 5 days to adjust to laboratory life before the experiment began [13]. The research fleas came from the neighborhood mutts. The animals were kept under typical laboratory settings (25°C 3°C, natural light, and 50-60% relative humidity). They ate a regular pellet meal and had access to an endless supply of water [14].

Plant materials

Plant specimens, including stems, leaves, and roots, of Tithonia diversifolia and Senna didymobotrya, were gathered in the Maragua region of Murang'a County. At the National Museums of East Africa, voucher

specimens were made and deposited after being photographed, identified, and authenticated by a taxonomist. The plants were taken to the Pharmacology and Toxicology labs at the Department of Public Health, where they were given a thorough washing under running water, cut into tiny pieces, allowed to air-dry in the shade for 14 days, and finally pulverized. At the University of Nairobi's Kabete campus, we used a previously published approach [15, 16] to extract plant components. Dimethyl sulfoxide (DMSO) and distilled water were each infused with 1000 mg/10 ml of the crude methanolic and aqueous extracts, respectively. Logarithmic serial dilutions were made in a sterile environment by adding

Using a concentration range of 100 mg/ml to 1 mg/ml, distilled water is added in a carefully measured quantity. All the crude extract solutions were kept at 40 degrees Celsius until they were needed.

Determination of in vitro anti-flea activity of *Tithonia diversifolia* and senna didymobotrya

As described before [17], the research employed a modified version of a contact assay using polypropylene (15 ml) centrifuge tubes to test for the presence of Ctenocephalides felis and Ctenocephalides canis. Herbalists often use water as a solvent for their potions, therefore methanolic crude extracts, which were discovered to be more active in piloting studies, were not investigated. The in-vitro anti-flea activity of Senna didymobotrya leaf extract (SLAE), Tithonia diversifolia leaf extract (TLAE), and Tithonia diversifolia flower extract (TFAE) was tested by preparing three different amounts of each crude aqueous extract. Three different strengths of each extract (1 mg/ml, 10 mg/ml, and 100 mg/ml) were prepared. After saturating a 10 cm by 1.5 cm strip of Whatman filter paper no.1 with the extract concentration in question and letting it dry, the paper was found to be uniformly coated with the extract at the measured concentration. The 15 ml polypropylene centrifuge tube was adapted to accommodate the coated stripe. In order to prevent the fleas from suffocating, 10 were selected at random and inserted into the polypropylene tubes and screw cap with holes.

Similar amounts of pyrethrum flower aqueous extract (PFAE) were used as a positive control. The fleas' viability in each tube was determined using the methods described by Dryden et al., [18]. Adult Ctenocephalides canis and felis fleas were collected from strays brought into the lab by Ndumboini residents. To keep the fleas contained, a huge container was filled with tiny bits of cotton wool. On average, there were 10 fleas in each tuft of cotton. Each tube now contains 10 fleas that were selected at random. To prevent the fleas from suffocating, an untreated screw cap with needle-punctured holes in the middle was used to seal the tubes. These tubes were stored horizontally at room temperature and relative humidity so that the fleas would have the most possible contact with the filter paper surface.

Acute dermal toxicity

Using OECD [19] recommendations 404, we evaluated the acute skin toxicity of the aqueous extracts. Nine mature female New Zealand albino rabbits weighing 2.0–3.0 kg were utilized for the experiment (three animals each test chemical). The test extract was topically administered to the rabbits at a dose of 2000 mg/kg. New Zealand albino rabbits were also used for eye irritation testing, as recommended by OECD standards 405.

Determination of acute toxicity levels of the active crude extract in rats

Wister rats and the OECD [13] technique, as outlined in guidelines 425, were used to determine the LD50 and acute toxicity of the crude aqueous extracts. Fifteen rats in all were used: three as controls, four for Senna didymobotrya leaf extracts, four for Tithonia diversifolia leaf extracts, and three for Tithonia diversifolia flower extracts.

Acute toxicity of active crude extracts was studied using groups of mature female nulliparous and non-pregnant Wister rats weighing 90-130 g.

Data Analysis

Data analysis was done using Student’s t-test, R version 3.4.3 and graphs were drawn using Microsoft Excel for 2010 year. The data obtained from In vitro anti-flea studies was expressed as a mean ± standard error of the mean (SEM) of the two independent experiments. The data from acute toxicity studies was analyzed qualitatively and quantitatively using suitable statistical tools. The LD_{50} values were calculated using the Acute Oral Toxicity Guidelines (425) Statistical Program Version: 1.0) [14].

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3. Results

The findings of the study are presented below

In Vitro Antifleas Activity of *Tithonia diversifolia* and *Senna didymobotrya* Crude Extracts in Comparison with Pyrethrum Flowers Aqueous Crude Extract.

Means of the aqueous extracts are shown in Table 1.

Extract	**Concentration**	**Means of dead fleas N_1**	**Means of life fleas N_{2*}**	**Initial Number of fleas N_2**	**Efficacy percentage $(N_2 - N_1)/ N_2 \times 100$**
TLAE	100 mg/ml	8.67	1.33	10	86.7
	10 mg/ml	5.33	4.77	10	53.3
	1 mg/ml	3.67	6.33	10	36.7
TFAE	100 mg/ml	9.33	0.77	10	93.3
	10 mg/ml	8.0	2.0	10	80
	1 mg/ml	3.67	6.33	10	36.7
SDLAE	100 mg/ml	6.63	3.37	10	66.3
	10 mg/ml	4.33	5.77	10	43.3
	1 mg/ml	4.0	6.0	10	40.0
PFAE	100 mg/ml	9.0	1.0	10	90
	10 mg/ml	7.33	2.67	10	73.3
	1 mg/ml	6.0	4.0	10	60.0

KEY: N2- Initial number of fleas; N1-Mean Number of Dead Fleas; N2*-Means of life fleas; TDFA-Tithonia diversifolia flowers aqueous extract; TDLA- *Tithonia diversifolia* leaves aqueous extract; SDLA-Senna didymobotrya leaves aqueous extract; PFAE-Pyrethrum Flowers aqueous extract

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Table 1: Means of fleas for all aqueous extract (TLAE, TFAE, SDLAE, and PFAE). Results of in vitro antifleas activity of all extracts in the study are compared and are shown in Figure 1.

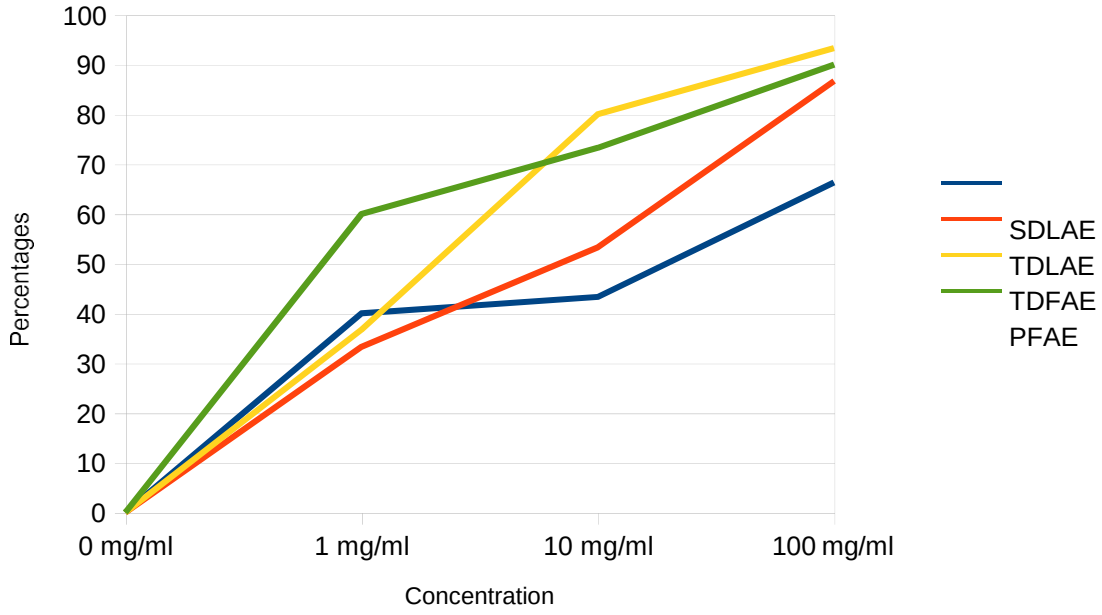

Figure 1: *In vitro* activity of SLAE, TDLAE, and TDFAE compared with PFAE extracts.

Acute Dermal Toxicity Profile of *Tithonia diversifolia* and *Senna didymobotrya* Crude Extracts in Newzealand Albino Rabbits

Acute dermal irritation: Effects of the extracts on the weight of the rabbits are shown in Table 2.

Rabbits	means weight in Kg at the beginning	means weight in kg at the end	p Value	Significant or not significant
TDFA	2.13	2.14	0.424	Not significant
TDLA	1.82	1.82	0.943	Not significant
SDLA	1.75	1.77	0.94	Not significant

Table 2: Rabbits mean weights before and 14 days after application of the extract.

Significant weight difference at 95% CI and $p \leq 0.05$

The effects of the crude extracts on the skin of the Newzealand albino rabbits are shown Table 3.

Crude Extract	Time after removal of the patch	0 minutes	60 minutes	24 hours	48 hours	72 hours

TDLA	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
TDFA	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
SDLA	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0

ERYTHEMA: 0-No erythema; 1-Very slight erythema (barely perceptible); 2-Well defined erythema; 3-Moderate to severe erythema; 4-Severe erythema (beef red); **OEDEMA:** 0-No oedema; 1-Very slight oedema, (barely perceptible); 2-Slight oedema, edges of the area well defined by definite rising; 3-Moderate oedema (raises approximately 1mm); 4-Severe oedema (raised more than 1mm and extending beyond the area of exposure).

Table 3: Dermal toxicity of crude aqueous extracts on Newzealand albino rabbit grading as per OECD 204.

Eye irritation test results for *Tithonia diversifolia* flowers aqueous crude extract, *Tithonia diversifolia* leaves aqueous crude extract, and *Senna didymobotrya* leave aqueous crude extract Results of eye irritation test are shown in Table 4.

Crude Extracts	**Cornea**	**Iris**	**Conjunctiva**	**Chemosis**
TDFA	0	0	0	0
TDLA	0	0	0	0
SDLA	0	0	0	0

CORNEA: 0-No ulceration or opacity was observed; 1-Scattered or diffuse areas of opacity; 2-Easily discernible translucent area; 3-Nacreous area (no details of iris visible); 4-Opaque cornea; **IRIS:** 0-Normal; 1-Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; 2-Hemorrhage, gross destruction or no reaction to light; **CONJUNCTIVA:** 0-Normal; 1-Some blood vessels hyperaemic; 2-Diffuse crimson color, individual vessels not easily discernible; 3-Diffuse beefy red; **CHEMOSIS:** 0-Normal; 1-Some swelling above normal; 2-Obvious swelling with partial eversion of the lids; 3-Swelling with lids about half closed; 4-Swelling with lids more than half closed.

Table 4: *T. diversifolia* Flowers aqueous extract eye irritation test results.

NB: No significant irritation was observed over the entire period of the study.

Effects of the crude extract on weight of the test animals: The effects aqueous crude extract on weight ofthe Wister rats are shown in Tables 5, 6 and 7. The effects T. diversifolia leaves aqueous (TDLA) extract on weight of the Wister rats are shown in Tables 5.

RAT	Weight in gram at the beginning	Dose in mg	Volume in ml	Weight in gram on day7	Weight in gram on 14
TDLA1	99.58	199.16	0.50	107.16	136.10
TDLA2	110.11	220.22	0.55	124.36	133.27
TDLA3	104.20	208.40	0.52	116.45	135.57
TDLA4	107.52	215.04	0.54	119.59	121.61
TDLA5	98.22	Control	Control	101.67	108.03

Table 5: Effects of T. diversifolia leaves aqueous (TDLA) on rat weights before and after oral administration of the crude extract to Wister rats.

$p = 0.01049$ at day 7. The value indicates that there is a significant weight difference at 95% CI at the beginning and on day 7; $p = 0.04384$ at day 14. The value indicates that there is a significant weight difference on day 14 for *Tithonia diversifolia* Leaves Aqueous crude extract. The effects of *Tithonia diversifolia* Flowers aqueous crude extracts on weight of the Wister rats are shown in Table 6.

RAT	Weight in gram at the beginning	Dose in mg	Volume in ml	Weight in gram on day 7	Weight in gram on day 14
TDFA1	100.00	200.00	0.50	122.07	134.44
TDFA2	100.27	200.54	0.51	115.07	118.34
TDFA3	98.90	197.80	0.49	113.54	124.03
TDFA4	102.29	204.58	0.511	115.92	126.13
TDFA5	97.34	Control	Control	105.13	108.85

Table 6: Effects of *T. diversifolia* Flowers Aqueous (TDFA) on rats' weights before and after oral administration of the crude extract to Wister rats.

Weight beginning and after day 7, p = 0.02222 at day 7.

The value indicates that there is a significant weight difference at 95% CI.

p = 0.02646 at day 14. The value indicates that there is a significant weight difference in the beginning and on day 14 for *Tithonia diversifolia* Flowers Aqueous crude extract. The effects of *Senna didymobotrya* Leaves Aqueous extract

(SDLA) on weights of the Wister rats are shown in Table 7.

RAT	Weight in gram at (day 0)	Dose in mg	Volume in ml	Weight in gram on day 7	Weight in gram on day 14
SDLA1	109.70	219.40	0.55	111.30	122.07
SDLA2	104.35	208.70	0.52	114.21	115.88
SDLA3	119.20	238.40	0.60	132.70	138.13
SDLA4	120.01	240.02	0.60	135.23	140.38
SDLA5	95.89	Control	Control	100.28	107.69

Table 7: Effects of *Senna didymobotrya* Leaves Aqueous extract (SDLA) on rat weights before and after oral administration of the crude extract.

Weight at the beginning and after 7 days for Senna didymobotrya Leaves Aqueous crude extract p = 0.1586 at day 7. The value indicates that there is no significant weight difference at 95 CI in the beginning. p = 0.1733 on day 14. The value indicates that there is no significant weight difference at 95% CI.

Acute LD_{50} results: The results of the limit test are summarized in Table 8. No rat died or was found moribund condition. One of the rats in *Senna didymobotrya* Leaves aqueous liter shown distress signs on the neck, but was stable up to the 14th day after drug administration. Another one in the same liter was wet with urine. The findings are shown in Table 8.

Extract Sample	Means of initial weight (g)	Means of weight (g) on day 7	Means weight (g) on day 14	mortality or moribund	Toxicity signs
TDFA	100.37 ± 1.41	116.65 ± 3.75	125.74 ± 6.67	0/4	Nil
TDLA	105.35 ± 4.55	116.89 ± 7.23	131.64 ± 6.80	0/4	Nil
SDLA	113.32 ± 7.60	123.36 ± 12.35	129.12 ± 12.01	0/4	Nil
Control (H_2O)	97.15 ± 1.20	102.36 ± 2.50	108.19 ± 0.60	0/3	Nil

TDLA- *Tithonia diversifolia* Leaves Aqueous; TDFA- *Tithonia diversifolia* Flowers Aqueous; SDLA-*Senna didymobotrya* Leaves Aqueous.

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Table 8: Acute Toxicity Results of Wister Rats.

One-Way Analysis of Variance (ANOVA) at 95 CI and level of significant being $p \leq 0.05$, $p = 0.2004$ the value indicates that there is no significant weight difference at long run.

Effects of the extracts on blood profile of the Wister rats:

The effects of the extracts on the blood profile are shown in the Tables 9.

Blood profile	**Crude extract**	**TDLA (4 rats)**	**TDFA (4 rats)**	**SDLA (4 rats)**	**Control (3 rats)**
RBCs (10^{12})	Means	6.76 ± 1.26	6.21 ± 2.15	5.27 ± 3.56	6.62 ± 0.75
	p Value	0.261	0.3604	0.504	
Hb (g/dl)	Means	15.83 ± 1.26	14.63 ± 2.15	13.68 ± 3.56	14.27 ± 0.75
	p Value	0.136	0.396	0.009	
Hct (Percentage)	Means	40.58 ± 9.13	36.55 ± 13.2	46.45 ± 4.67	37.46 ± 4.76
	p Value	0.136	0.196	0.026	
MCV(FI)	Means	59.73 ± 4.36	58.95 ± 3.99	64.18 ± 3.17	56.63 ± 1.29
	p Value	0.195	0.699	0.013	
MCH (pg)	Means	21.73 ± 0.92	21.95 ± 0.54	21.98 ± 2.32	21.50 ± 0.79
	p Value	0.092	0.620	0.921	
MCHC (g/dl)	Means	36.50 ± 2.72	37.40 ± 2.29	34.33 ± 3.41	37.97 ± 1.48
	p Value	0.892	0.831	0.054	
RDW	Means	20.20 ± 0.71	19.08 ± 1.48	21.35 ± 1.02	20.70 ± 0.30
	p Value	0.109	0.115	0.161	
PLTs (K/ µL)	Means	475.50 ± 295	471.00 ± 195	648.00 ± 299	381.67 ± 298.63
	p Value	0.059	0.416	0.393	
WBCs (K/ µL)	Means	7.65 ± 3.84	10.12 ± 5.24	10.30 ± 2.11	10.58 ± 3.00
	p Value	0.855	0.917	0.042	
N(Percentages)	Means	27.40 ± 19.20	38.48 ± 8.69	27.43 ± 18.04	49.93 ± 12.3
	p Value	0.027	0.843	0.020	
L($\times 10^9$)	Means	5.61 ± 3.63	6.03 ± 4.13	6.74 ± 0.37	4.78 ± 1.66
	p Value	0.228	0.862	0.016	
M($\times 10^9$)	Means	0.21 ± 0.13	0.26 ± 0.23	0.27 ± 0.03	0.28 ± 0.03
	p Value	0.301	0.751	0.205	
E($\times 10^9$)	Means	0.12 ± 0.05	0.17 ± 0.12	0.17 ± 0.07	0.20 ± 0.14
	p Value	0.119	0.053	0.082	

B($\times 10^9$)	Means p Value	0.02 ± 0.01 0.391	0.015 ± 0.017 0.604	0.020 ± 0.01 0.18	0.10 ± 0.00

The extracts were compared with the control with level of significant being $p \leq 0.05$ at 95% CI,

RBCs-Red Blood Cells; Hb (g/dl)-Haemoglobin; Hct - Hematocrit; MCV (FI)-Mean Corpuscular Volume; MCH-Mean

Cell Hemoglobin; MCHC-Mean Cell Hemoglobin Concentration; RDW-Red blood cells distribution width; PLT-

Platelets; WBC-White Blood Cells; N-Neutrophils; L-Lymphocytes; M-Monocytes; E-Eosinophil; B-Basophils.

Table 9: Effects of the crude extracts on Red Blood Cells (RBCs) 14 days after oral administration to Wister rats.

4. Discussion

Below, we compare and contrast the two plants in terms of their effectiveness and safety as flea repellents.
The aqueous extract (TFAE) of flowers from the Tithonia diversifolia plant showed the highest level of anti-flea action. In comparison to pyrethrum flowers aqueous extract (PFAE), which killed 90.0% and 73.3% of fleas at the same doses in 24 hours, TFAE was 93.3 percent effective at 100 mg/ml and 80.0 percent effective at 10 mg/ml. Within 24 hours, 86.7% of fleas were eliminated by the Tithonia diversifolia leaves aqueous extract (TLAE), whereas 66.3% and 43.3% were destroyed by the Senna didymobotrya leaves aqueous extract (SLAE). These results are consistent with those found by Adayo et al. [11] and with its effectiveness in reducing jigger populations in Kenya's Ikolomani Division, Kakamega County [12]. The research, however, was conducted only on the Tithonia diversifolia leaves. In this study, researchers discovered that flowers were the most effective against fleas.

Two thousand milligrams per kilogram of Tithonia diversifolia aqueous extracts were utilized in acute oral toxicity testing. Since there was no substantial oral acute toxicity based on its influence on weight and hematological profile (with the exception of Neutrophils), and no death within 24 hours, the LD50 of the extracts was higher than the amount employed. Ezeonwumelu et al. [20] and Kamatenesi-Mugisha et al. [22] also report results that are consistent with these ones.

According to [21], an aqueous extract of Tithonia diversifolia leaves showed an LD 50 of above 10,000 mg/kg.
Elufioye et al. [22] employed 400-1600 mg/kg of ethanoic extract and found no evidence of acute toxicity, as shown by the lack of hematological alterations. When given to cockerels for 98 days, Funmilayo et al. [23] reported no significant harmful effects on hematological or biochemical markers.

The present research indicated that unlike S. didymobotrya, Tithonia diversifolia had an influence on weight increase in Wister rats, with its leaves having a value of $p = 0.0104$ at day 7 and $p = 0.044$ at day 14, and its flowers having a value of $p = 0.0222$ at day 7 and $p = 0.0265$ at day 14. These results corroborate those of Mauricio et al., [24], who found that T. diversifolia might be useful in animal diets.

Tithonia diversifolia aqueous extract had an intra-peritoneal LD50 of 100 mg/kg when given once daily for 14 days, however Oyewole et al. [25] discovered that this dosage was 120 mg/kg. He claimed substantial weight changes, hematological abnormalities, and increased mortality, all of which were not seen in our research. It's probable that the several doses and intra-peritoneal delivery account for this variation. The crude aqueous extracts of Tithonia diversifolia leaves and flowers caused little acute cutaneous irritation and minimal ocular discomfort.

The LD50 for an acute oral toxicity test using Senna didymobotrya leaf aqueous extract was determined to be more than 2000 mg/kg body weight. There were no reported deaths or significant weight loss in the animals, but there were significant acute toxic effects observed, as indicated by the hematological changes on the Hemoglobin ($p = 0.009$), Hematocrit ($p = 0.026$), Mean Cell Volume ($p = 0.013$), White Blood Cells ($p = 0.042$), Neutrophils ($p = 0.0204$), and Lymphocytes ($p = 0.016$). In addition, there was no evidence of severe acute skin toxicity or corrosion, or considerable ocular irritation or corrosion. The LD50 for Senna didymobotrya DCM extract was determined to be between 1000 and 5000 milligrams per kilogram of body weight by Korir et al. [26]. Acute toxic effects were not seen at lower dosages,

however weight loss revealed that toxic effects increased dramatically beyond 3000 mg per kg body weight and with ongoing daily administration. Similar results were found by Nyamwamu et al. [27] for Senna didymobotrya crude root extracts in methanoic and Dichloromethane, and for extracts in hexane and water, with an LD50 of 1927 mg/kg and over 5000 mg/kg, respectively, in mice. Ingestion of an aqueous extract poses only a little health risk. This study's findings on the safety of Senna didymobotrya extracts for topical treatment are consistent with those of Nyamwamu et al. [27] and Njoroge et al. [28] regarding their usage in the management of human and cattle cutaneous disorders and ectoparasites.

Blood profile was not substantially altered by Tithonia diversifolia flowers aqueous (TDFA), making it the extract of choice for this investigation. In terms of its antiflea action, it was likewise determined to be the most effective, showing striking similarities to the positive control (pyrethrum flowers aqueous extract). Tithonia diversifolia (TDLA) leaves aqueous extract exhibited significantly less activity than flowers but more activity than Senna didymobotrya (SDLA) leaves aqueous extract. Also, only neutrophils ($p = 0.027$) exhibited substantially reduced numbers compared to control Wister rats, suggesting that it was not particularly harmful to the blood cells.

5. Conclusion

The research were able to determine the following. The In vitro anti-flea activity of aqueous extracts of C. cinerariifolium (pyrethrum) flowers, T. diversifolia leaves and flowers ($p=0.8321$), and S. didymobotrya leaves ($p=0.8321$) was not significantly different from that of the other extracts.

More research on the pesticidal properties of Tithonia diversifolia flowers is required.

6. Recommendations

The research led to the following suggestions.

To better purify useable pesticides that are cost efficient, particularly in controlling the jigger issue in Kenya and elsewhere, more research has to be done on Tithonia diversifolia and Senna didymobotrya extracts. The blossoms of the Tithonia diversifolia plant need further attention since they have been shown to be both effective and safe. The floral extracts might deliver safe, cheap, and effective insecticides, but further research is needed to refine the process. Finally, people in Murang'a and elsewhere in Kenya might learn from the success of Kakamega herbalists who use a cooked Tithonia diversifolia combination to clean jigger infected regions of the body.

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