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Toxicological Investigations of Plants Traditionally used for Mosquito Control in Kenya's South Coast

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ABSTRACT

Background: Mosquito borne diseases affect many people globally and impede economic development. Plants are used for mosquito control since they are cost-effective, readily available and culturally acceptable.
Objective: To investigate toxicological effects of plants use for mosquito control in Kenya's south coast.
Methods: Aqueous, acetone and hexane extract of *L. camara* (leaves), *T. minuta* (whole plant), *A. indica* (leaves), *A. digitata* (leaves), *O. suave* (whole plant) and *P. barbatus* (leaves) were tested for genotoxic potential using *Allium cepa* test. Acute dermal irritation and ocular toxicity were conducted as per OECD test guidelines.
Results: There were significant differences among extracts and concentrations $p<0.001$. *A. indica* extracts showed a significant difference on root growth; *P* value for hexane was < 0.001, aqueous, 0.004 and acetone, 0.007. The roots were bent. *A. indica* showed most mitotic inhibition at concentration of 1mg/ml at 70.36±0.68 which was similar to vincristine sulphate. The least active was *A. digitata* at 1.42±0.68 at 0.125 mg/ml. Extracts of *O. suave* caused turgidity on the roots of *Allium cepa. A. indica* extracts had ghost cells(aqueous) and binucleate cells (acetone) while Aqueous extract of *P. barbatus* showed high cytoplasm to nucleus ratio.
Conclusion: *A. indica* and *P. barbatus* extracts caused abnormal cells and it is possible for them to cause the same in human. None of the extracts exhibited dermal irritation or acute ocular toxicity. There was no significant change in weight of the test animals during the treatment period and there were no mortalities.

Key words: genotoxic potential, *Allium cepa* test, mitotic inhibition, acute dermal irritation/corrosion, ocular toxicity

INTRODUCTION

Mosquitoes are nearly ubiquitous and inhabit most regions except Antarctica existing in regions more than five thousand metres above sea level and one thousand three hundred metres below sea level. Mosquitoes are of major public health concern since they transmit diseases [1] which contribute to mortality and poverty and thereby stifling socio-economic development in tropical and subtropical countries. [2] The diseases infect over a billion people worldwide annually with resultant many deaths. [3,4] Malaria kills almost two million people annually worldwide [5] while there is a resurgent of Dengue virus hemorrhagic fever and West Nile virus. Chikungunya virus affects millions in Indian Ocean basin while Japanese encephalitis is endemic in the Indian subcontinent and Australasia. In

addition, filariasis is currently a subject of a global eradication campaign. [6] Rift Valley Fever is prevalent in Africa and the Middle East where it has caused illnesses in human and domestic animals. [7]

Mosquito bites also cause considerable annoyance, can lead to allergic reactions, dermatitis and secondary infections. [8] Plants have been used since antiquity all over the world for control of mosquitoes. [9] The aim of this study was to assess potential toxicity of plants used for mosquito control in Kwale county of Kenya's south coast.

The plants for this study were selected on available ethnomedicinal information [10] and review of relevant literature on their use for control of mosquitoes. The six plants and their parts are shown in table 1 and were *Lantana camara* (leaves), *Tagetes minuta* (whole plant), *Azadirachta indica* (leaves), *Adansonia digitata* (leaves), *Ocimum suave* (whole plant) and *Plectranthus barbatus* (leaves). Plants were collected after initial field identification with the aid of traditional herbal practitioners from Msambweni Sub County. Further identification was done by a plant taxonomist at the Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi where voucher specimens were deposited.

Harvesting of the plants' parts was done on the months of September and November when there is adequate foliage following the rains and material of best quality is ensured. [11] The harvested plant parts were first cleaned with water then dried off the water and stored in dry sacks. The material was then taken to the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. The plants were tested for genotoxic potential, acute dermal irritation /corrosion and acute ocular irritation using established protocols.

The purpose of genotoxicity as being to recognize possible mutagenic dangers of substances to human and defining the mechanism of action of those that have that capability. [12] The tests are of various types and include those conducted using organisms or simulations of body structures and those that are conducted outside organisms. According to OECD, [13] this testing is necessary in order to project possible cancer causes and study how they come arise.

During assessment and evaluation of substances, any material that is going to be applied on human skin must be assessed for irritability and corrosion potential through the acute dermal irritation/corrosion test. This is done to determine the degree of irritation that a dilution of a test material can produce on the skin of New Zealand white rabbit, usually three per dilution of test substance. [14]

It avails information on possible dangers from short-term exposures through the skin and also provides information on absorption through the skin and the mode of toxic action of a substance by topical route. It is an initial part in determining a dosage regimen for subsequent studies. [15,16] To guarantee safety and care of the test animals for the acute dermal and ocular irritation testing, the study protocol was submitted to and approval obtained from the Faculty of veterinary medicine biosafety, animal use and ethics committee of the University of Nairobi.

Products being applied to the skin especially on the face should also be evaluated for their effects on the eyes. Draize testing [20] is the major test for determination of toxicity to the eyes. [17-19] It uses New Zealand white rabbits due to the advantage with their eyes having excellent anatomy and physiology. [21]

MATERIALS AND METHODS

Water extraction

One kilogram (1000 grams) of each plant powder was extracted separately with water in conical flasks in which frequent stirring of the mixture was done to ensure proper mixing. The conical flasks were corked tightly with stoppers. Shaking was done regularly to allow for maceration for four days. On the fifth day filtration was done using Whatman No.1 filter paper. The filtrate was stored in sterilized beakers, covered tightly with aluminum foil and stored in a refrigerator at $^{+}4^{0}$ C pending

freeze-drying. Freeze drying was done using Virtis Bench Top 3® Model freeze drier (The Virtis Company, New York), at the Department of Veterinary Anatomy and Physiology, University of Nairobi. The freeze-dried material was used for subsequent tests.

Acetone extraction

One kilogram (1000 grams) of each plant powder was extracted separately with analytical grade acetone in conical flasks. To ensure mixing, stirring was done frequently and then the conical flask corked with appropriate stopper. Thereafter, shaking was done regularly to allow maceration. On the fifth day, the extracts were filtered using Whatman No.1 filter paper into another conical flask. Acetone was removed in a rotary evaporator at 40^0C and the substance obtained was allowed to dry off the acetone then stored in $^+4^0$C pending laboratory tests.

Extraction using hexane

One kilogram (1000 grams) of the plant powder was extracted with hexane in a conical flask and then the conical flask corked with appropriate stopper. Stirring was done to ensure proper mixing and percolation and on the fifth day filtration was done with Whatman No.1 filter paper. Hexane was removed in a rotary evaporator at 40^0C and the substance obtained dried then stored in amber coloured bottle in a refrigerator at $^+4^0$C pending laboratory tests.

Genotoxic testing

The *Allium cepa* test

This was performed according to established method.[22] Sixteen (16) *Allium cepa* bulbs (2.5–2.8 cm diameter) were used per concentration per test sample. They were grown in small cups with water at room temperature for 3 days for emergence of roots. Root lengths were measured periodically and scored. When roots lengths were 3 cm, the bulbs were treated with the extracts and the controls at concentrations of 125, 250, 500, and 1,000 µg/ml. Positive control was vincristine sulphate and negative control was tap water. [23]

Roots were assessed for root length, turgescence, form and colour change. About 2 mm of the root tips were fixed in ethanol: glacial acetic acid (3:1 v/v) and hydrolyzed in 1N HCL at 60 °C for five minutes then cleaned with distilled water. They were compacted on a microscope slide and stained with aceto-orcein for 10 min. Excess stain was removed and cover slips placed on the smear. Cover slips were sealed on the slides with clear fingernail polish. [24]

Observation of the slides was at 40× magnification under a light microscope. Photomicrographs were made and analyzed for mitotic index, early anaphases, chromosomal bridges/fragments, stickiness and c-mitosis. Mitotic index was calculated as = Number of cells in mitosis/Total number of cells. [23, 25]

STATISTICAL ANALYSIS

Data obtained was analyzed using the SPSS V26 for means, standard deviations of means followed by one-way ANOVA and Student Newman Keul (SNK) test. Results with $P< 0.05$ were considered significant.

Acute dermal irritation testing

In assessment and evaluation of substances on human skin, the acute dermal irritation/corrosion test is necessary. This is done to determine the degree of irritation that a dilution of a test material can produce on the skin of New Zealand white rabbit, usually three per dilution of test substance.[14] It provides information on absorption and possible risks including the mode of toxic action of a substance by topical route from short-term exposures through the skin. It is an initial part in determining a dosage regimen for subsequent studies.[15,16] To guarantee safety and care of the test animals for the acute dermal, the study protocol was submitted to and approval obtained from the Faculty of Veterinary Medicine Biosafety, Animal Use and Ethics Committee of the University of Nairobi.

The test animals

The New Zealand white rabbit is an appropriate model for this study since the results can be easily compared to other data bases and extrapolated to human.[26] Three New Zealand white rabbits per plant extract per concentration were used. They weighed 2.5- 3 kg and aged 18-20 weeks. They were housed individually in the animal house at the Department of Public Health, Pharmacology and Toxicology of the University of Nairobi in relative humidity of 50-60% and lighting simulating day and night with conventional laboratory diet and unrestricted access to water.[14,27]

The procedure for Acute dermal irritation testing

This was performed on intact and abraded skin of rabbits. Only animals with healthy intact epidermis by gross observation were used for the study, and three rabbits were used per test. For intact skin, prior to the test, fur was removed through shaving the left and right dorsal areas of the trunk of the animals. The skin was cleaned with distilled water and left for 24 hours. This was to allow for recovery of the *stratum corneum* from any disturbance caused by the shaving. [28] Testing on abraded skin was done to simulate situations when the skin has wounds, pimples or scratches. The same procedure as for the intact skin was used except that the shaved skin was rubbed with a fine abrasive paper.[29]

Half a milliliter (0.5 ml) of each extract was spread evenly to about 6 cm^2 of skin on the left dorsal area. It was covered with a gauze patch held in place by a non-irritating tape. The shaved skin on the right side applied with only 0.5 ml of distilled water was the control. Trunks of the test animals were wrapped with corsets to prevent them from interfering with the patches. After four (4) hours, the extracts were cleaned off by gentle swabbing with cotton wool soaked in distilled water. The animals were observed for signs of irritation such as erythema and oedema. Findings were scored at 1 hr, 24 hrs, 48 hrs and 72 hours after patch removal. The animals were further observed for any signs of dermatoxicity, behavior, general condition, posture and reflexes, attitude towards food, water, and hygiene on days 7 and 14 [14] They were weighed on day 0 and the last day of the experiments.

Determination of Primary Irritation Index

The primary irritation index (PII):

PII = Σ (erythema grade at 24, 48 and 72 hr) + Σ (oedema grade at 24, 48 and 72 hr)/ total number of observations. [30]

Ocular toxicity testing

Plants' extracts were prepared into 1, 10, and 100 µg/ml then used for this test. Three New Zealand white rabbits were used per extract per concentration. Only rabbits with non-deformed eyes were used. A tenth of a millimeter of test substances was deposited in one eye and lids held together for one minute then left for twenty four hours. The other eye served as control. Observations were made and recorded at 1, 24, 48, and 72 hours days 7, 14 and 21 following test substance application. The animals were observed and any redness, swelling, cloudiness, oedema, hemorrhage, discharge, excessive blinking, excessive tearing, corneal damage, absence of light reflex and conjunctival ulceration and blindness recorded. [31,32] The observations were recorded and graded according to OECD guidelines. [31]

RESULTS

Genotoxic testing

Root length

The effects of *L. camara* crude extracts on *Allium cepa* root growth are shown on table 2. Root length was dependent on dose with the highest concentration (1 mg/ml) showing least root growth. Significance difference did not exist on root growth among the different concentrations of *Tagetes minuta* extracts, table 3 below. Root growth was dose dependent. Among the different concentrations of *Azadirachta indica* as shown on table 4, there was significant

difference on root growth; *P* value for hexane was less than 0.001 that of aqueous extracts was 0.004. There was significant difference on the dose versus root growth for acetone extracts as the *P* value was 0.007. The roots were bent but there was no colour change among the *Azadirachta indica* extracts. Root growth was dose dependent.

The effects of *Ocimium suave* extracts are shown on table 5. There was no significant difference on root growth among the different extracts and the concentrations. Extracts of *Ocimium suave* caused roots of *Allium cepa* to be turgid. For *Adansonia digitata*, there was significant difference on root growth in different doses of the extracts, table 6. However, there was no significant difference in root growth inhibition across the concentrations. Among the extracts of *Plectranthus barbatus*, there was no significant difference on root growth across the extracts, table 7. Fig 1 Shows examples of observations on the roots. The mean root lengths of different plant extracts in relation to various concentrations are shown figures below. Fig 2 represents acetone extracts, Fig 3 hexane extracts and Fig 4, aqueous extracts.

Table 1: Plants selected for the study

Family	Plant species, voucher specimen	Local name	Life form	Part used
Asteraceae	*Tagetus minuta* L. (JM 17)	Bangi ya shambani	Herb	Whole plant
Bombacacea	*Adansonia digitata* Linn. (JM 09)	Mbuyu / Mbamburi	Tree	Leaves
Labiatae	*Ocimum suave* Willd (JM 05)	Kirihani/Kivumbani	Herb	Whole plant
Labiatae	*Plectranthus barbatus* Andr. (JM 03)	Kizimwilo	Shrub	Leaves
Meliaceae	*Azadirachta indica* (L) Burm. (JM 10)	Mwarobaini/ Mkilifi	Tree	Leaves
Verbenaceae	*Lantana camara L* (JM 11)	Mjasasa	Shrub	Leaves

Vincristine sulphate ***A. indica*** **Tap water** ***O. suave***

Fig 1: Effects of some of the plants' extracts and controls on *Allium cepa* roots

Table 2: Mean root lengths of extracts of *Lantana camara*

Extract/ Concentration	Acetone extract Mean±SE	Hexane extract Mean±SE	Aqueous extract Mean±SE	P-value
0.125 mg/ml	4.36 ± 0.16^{c}	4.50 ± 0.13^{d}	4.42 ± 0.17^{c}	0.812
0.25 mg/ml	4.08 ± 0.15^{c}	4.00 ± 0.13^{c}	4.14 ± 0.14^{c}	0.785
0.5 mg/ml	3.66 ± 0.06^{b}	3.58 ± 0.07^{b}	3.70 ± 0.09^{b}	0.553
1 mg/ml	2.92 ± 0.07^{a}	2.98 ± 0.07^{a}	3.00 ± 0.08^{a}	0.711
	P<0.001	*P*<0.001	*P*<0.001	

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

Table 3: Mean root lengths in various extracts of *Tagetes minuta*

Extract/ concentration	Acetone extract Mean±SE	Hexane extract Mean±SE	Aqueous extract Mean±SE	P-value
0.125 mg/ml	4.80 ± 0.07^{c}	4.72 ± 0.09^{c}	4.82 ± 0.07^{b}	0.619
0.25 mg/ml	4.40 ± 0.16^{b}	4.32 ± 0.15^{b}	4.44 ± 0.23^{b}	0.895
0.5 mg/ml/	4.26 ± 0.17^{b}	4.18 ± 0.16^{b}	4.30 ± 0.17^{b}	0.874
1 mg/ml	3.52 ± 0.10^{a}	3.36 ± 0.11^{a}	3.58 ± 0.13^{a}	0.393
P-value	<0.001	<0.001	<0.001	

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

Table 4: Means of root lengths of various *Azadirachta indica* extracts

Extract/ Concentration	Acetone extract Mean±SE	Hexane extract Mean±SE	Aqueous extract Mean±SE	P-value
0.125 mg/ml	4.16 ± 0.17^{c}	4.22 ± 0.12^{b}	4.28 ± 0.15^{c}	0.850
0.25 mg/ml	3.86 ± 0.17^{bc}	3.96 ± 0.13^{b}	3.96 ± 0.12^{bc}	0.855
0.5 mg/ml/	3.38 ± 0.18^{ab}	3.54 ± 0.19^{a}	3.48 ± 0.20^{ab}	0.837
1 mg/ml	3.12 ± 0.24^{a}	3.14 ± 0.10^{a}	3.20 ± 0.25^{a}	0.960
p-value	P=0.007	p<0.001	P=0.004	

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

Table 5: Root length in various doses and extracts of *Ocimum suave*

Extract/ concentration	Acetone extract Mean±SE	Hexane extract Mean±SE	Aqueous extract Mean±SE	P-value
0.125 mg/ml	4.32 ± 0.06^{b}	4.20 ± 0.07^{c}	4.38 ± 0.14^{b}	0.442
0.25 mg/ml	4.10 ± 0.04^{b}	4.02 ± 0.07^{c}	4.16 ± 0.07^{b}	0.326
0.5 mg/ml/	3.62 ± 0.16^{a}	3.46 ± 0.14^{b}	3.66 ± 0.18^{a}	0.655
1 mg/ml	3.32 ± 0.11^{a}	3.14 ± 0.10^{a}	3.36 ± 0.10^{a}	0.318
p-value	<0.001	<0.001	<0.001	

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

Table 6: Means of various extracts of *Adansonia digitata*

Extract/ concentration	Acetone extract Mean±SE	Hexane extract Mean±SE	Aqueous extract Mean±SE	*P*-value
0.125 mg/ml	4.92 ± 0.19^{b}	5.08 ± 0.15^{c}	-	0.563
0.25 mg/ml	4.62 ± 0.28^{b}	4.60 ± 0.24^{bc}	4.78 ± 0.26^{b}	0.869
0.5 mg/ml/	4.36 ± 0.22^{ab}	4.28 ± 0.20^{ab}	4.52 ± 0.19^{b}	0.709
1 mg/ml	3.82 ± 0.16^{a}	3.74 ± 0.15^{a}	3.90 ± 0.10^{a}	0.725
p-value	0.016	0.002	0.023	

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

Table 7: Means of various extracts of *Plectranthus barbatus*

Extract/ Concentration	Acetone extract Mean±SE	Hexane extract Mean±SE	Aqueous extract Mean±SE	P-value
0.125 mg/ml	4.92 ± 0.18^{b}	5.02 ± 0.16^{c}	4.88 ± 0.19^{b}	0.845
0.25 mg/ml	4.40 ± 0.19^{b}	4.10 ± 0.10^{b}	4.64 ± 0.19^{b}	0.106
0.5 mg/ml/	4.34 ± 0.19^{b}	3.86 ± 0.33^{b}	4.30 ± 0.20^{b}	0.349
1 mg/ml	3.00 ± 0.06^{a}	3.08 ± 0.13^{a}	3.14 ± 0.11^{a}	0.657
	P<0.001	P<0.001	<0.001	

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

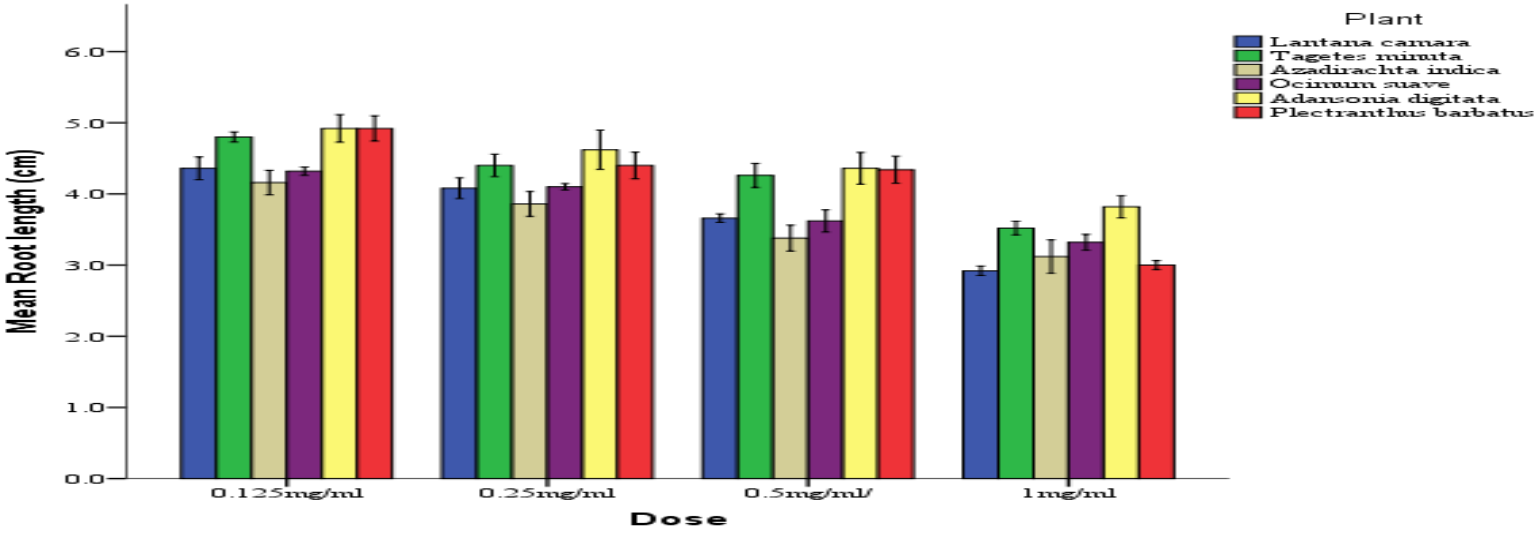


Figure 2: Means of root lengths in various acetone extracts

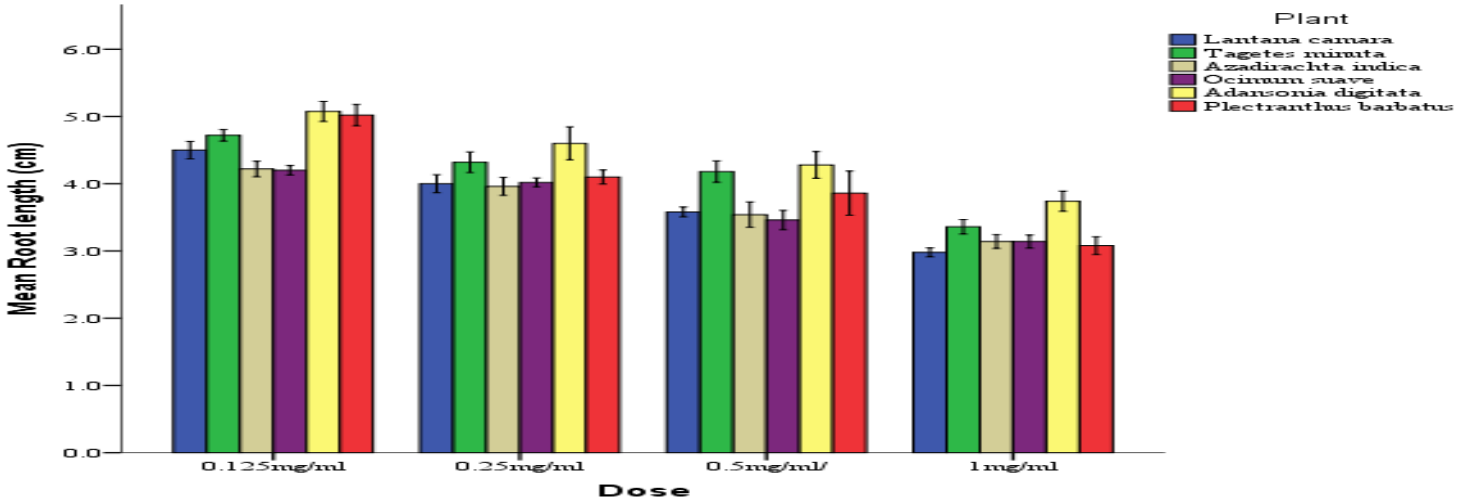


Figure 3: Means of root lengths in various hexane extracts

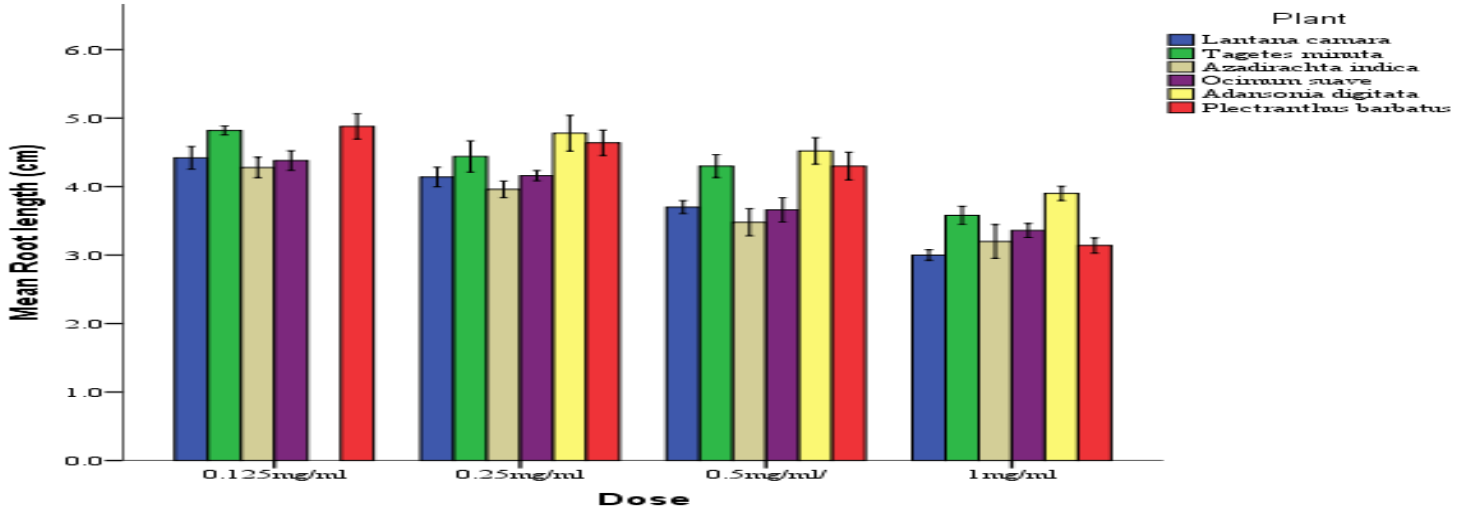


Figure 4: Means of root lengths in various Aqueous extracts

Assessment of damage to chromosomes

Fig 5 below shows some of the effects induced by different extracts on the *Allium cepa* meristem cells. Among the acetone extracts, as shown in table 8, there existed a difference in activity among the plants. *Azadirachta indica* showed most mitotic inhibition at concentration of 1mg/ml at 70.36±0.68 which was similar to vincristine. In table 9 the mitotic inhibition effects of hexane extracts are shown. The most active was *Azadirachta indica* at 1 mg/ml at 71.53±1.18 which was not significantly different from vincristine sulphate while the least active was *Adansonia digitata* at 1.42±0.68 at 0.125 mg/ml. There were significant differences among extracts and concentrations p<0.001.

For aqueous extracts (table 10), the most active was *A. indica* at 1mg/ml at a value of 67.95±0.72 which was not significantly different from vincristine sulphate. The least active was *Adansonia digitata* at concentration of 0.125 mg/ml with a value of 2.66±0.76.

Table 8: Mitotic inhibition of acetone extracts

Plant/Treatment	Concentration			
	0.125 mg/ml	0.25 mg/ml	0.5 mg/ml	1 mg/ml
Lantana camara	23.55 ± 0.79^{Ad}	40.76 ± 0.93^{Be}	50.36 ± 0.66^{Cd}	62.45 ± 1.10^{De}
Tagetes minuta	-	27.49 ± 0.68^{Ad}	33.80 ± 0.63^{B}	44.93 ± 0.95^{Cd}
Azadirachta indica	28.91 ± 0.98^{Ae}	41.06 ± 0.92^{Be}	54.57 ± 0.67^{Ce}	70.36 ± 0.68^{Df}
Ocimum suave	13.76 ± 0.51^{Ab}	23.40 ± 1.17^{Bc}	36.01 ± 2.21^{Cc}	37.83 ± 0.70^{Cc}
Adansonia digitata	1.02 ± 0.46^{Aa}	16.27 ± 1.02^{Bb}	23.62 ± 1.02^{Cb}	29.53 ± 0.61^{Db}
Plectranthus barbatus	18.61 ± 2.33^{Ac}	23.59 ± 1.24^{Bc}	33.55 ± 0.73^{Cc}	45.16 ± 1.14^{Dd}
tap water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
vincristine sulphate	94.99 ± 0.38^{f}	94.99 ± 0.38^{f}	94.99 ± 0.38^{f}	94.99 ± 0.38^{f}
	P<0.001	P<0.001	P<0.001	P<0.001

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

Table 9: Mitotic inhibition of hexane extracts

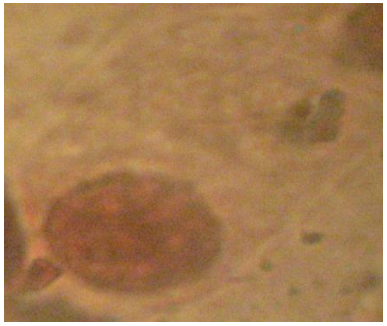
Treatment	Concentration			
	0.125 mg/ml	0.25 mg/ml	0.5 mg/ml	1 mg/ml
Lantana camara	20.02 ± 1.26^{Ac}	27.46 ± 1.07^{Bcd}	53.16 ± 1.10^{Ce}	63.79 ± 0.73^{De}
Tagetes minuta	22.10 ± 0.84^{Ac}	23.85 ± 0.86^{Ac}	$33.95\pm0.82B^{c}$	43.49 ± 0.84^{Cc}
Azadirachta indica	29.64 ± 0.84^{Ad}	34.60 ± 1.17^{Be}	57.30 ± 1.47^{Cf}	71.53 ± 1.18^{Df}
Ocimum suave	17.13 ± 0.71^{Ab}	28.08 ± 1.81^{Bcd}	37.66 ± 1.23^{Cd}	48.27 ± 0.87^{Dd}
Adansonia digitata	1.42 ± 0.68^{Aa}	17.49 ± 1.34^{Bb}	25.72 ± 1.16^{Cb}	30.74 ± 0.91^{Db}
Plectranthus barbatus	21.39 ± 0.46^{Ac}	30.92 ± 1.91^{Bd}	36.06 ± 1.11^{Ccd}	42.61 ± 1.31^{Dc}
tap water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
vincristine sulphate	94.99 ± 0.38^{e}	94.99 ± 0.38^{f}	94.99 ± 0.38^{g}	94.99 ± 0.38^{g}
p-value	< 0.001	< 0.001	< 0.001	< 0.001

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

Table 10: Mitotic inhibition of aqueous extracts

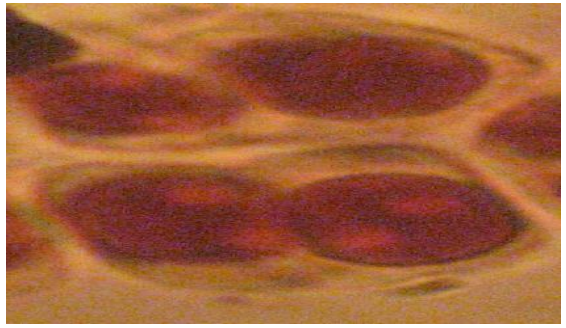
Treatment	Concentration			
	0.125 mg/ml	0.25 mg/ml	0.5 mg/ml	1 mg/ml
Lantana camara	18.43 ± 0.98^{Ab}	34.31 ± 0.78^{Bd}	44.00 ± 1.41^{Cd}	61.53 ± 0.49^{De}
Tagetes minuta	17.70 ± 0.98^{Ab}	22.90 ± 1.44^{Bc}	35.95 ± 1.06^{Cc}	40.51 ± 1.61^{Dc}
Azadirachta indica	28.93 ± 1.54^{Ac}	40.46 ± 0.91^{Be}	51.36 ± 0.81^{Ce}	67.95 ± 0.72^{Df}
Ocimum suave	16.51 ± 0.50^{Ab}	22.57 ± 1.60^{Bc}	35.85 ± 0.80^{Cc}	38.44 ± 2.20^{Cc}
Adansonia digitata	2.66 ± 0.76^{Aa}	15.89 ± 1.36^{Bb}	22.38 ± 1.59^{Cb}	25.64 ± 1.71^{Cb}
Plectranthus barbatus	19.70 ± 1.75^{Ab}	25.04 ± 1.07^{Bc}	34.42 ± 0.79^{Cc}	46.45 ± 0.67^{Dd}
tap water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
vincristine sulphate	94.99 ± 0.38^{d}	94.99 ± 0.38^{f}	94.99 ± 0.38^{f}	94.99 ± 0.38^{g}
P-value	< 0.001	< 0.001	< 0.001	< 0.001

Values of mean followed by similar letter/ capital letter(s) in the same row are not significantly different from one another (One –way ANOVA, SNK-test, α=0.05)

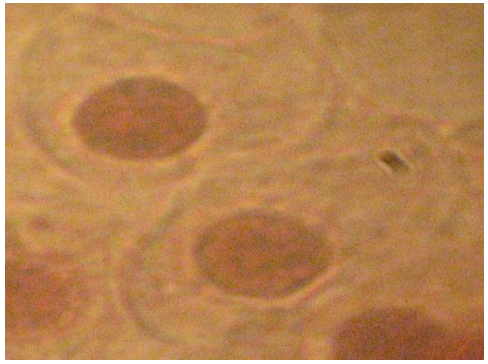


Ghost cell (*A.indica* aqueous extract)

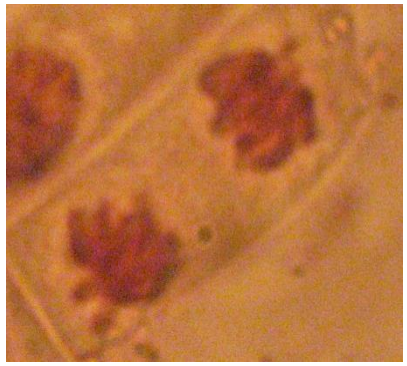
Binucleate cells (*A.indica* acetone extract)



Binucleate cells due to vincristine



High cytoplasm to nucleus ratio (Aqueous extract of *P. barbatus*)

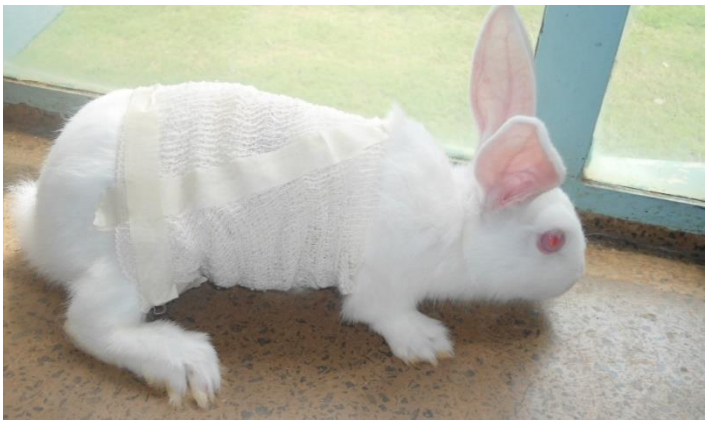


Normal metaphase

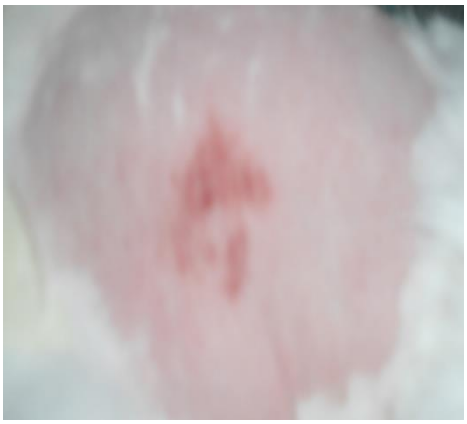
Fig 5: Examples of observed effects of plant extracts and controls

The acute dermal irritation test

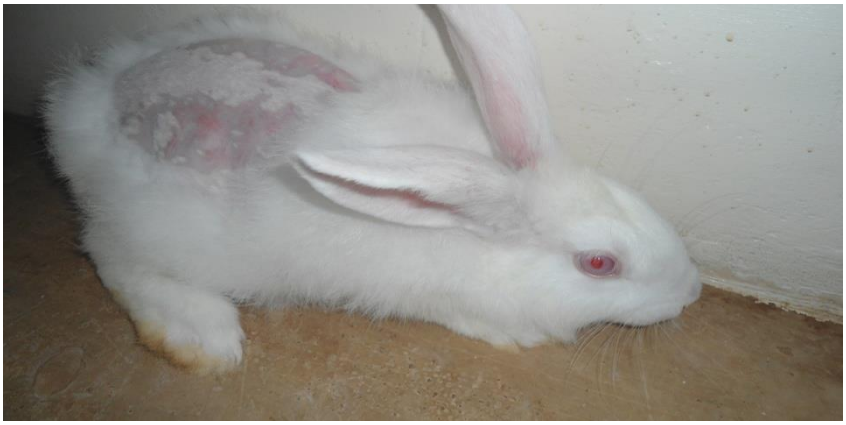
Fig 6 below shows one of the test animals that had just been prepared for the tests using an improvised corset. It also shows an abraded skin area of a rabbit and the rabbit skin healing well. The results showed that the tested six plants did not cause any toxicity even after 14 days of observation. There were no signs of acute dermal toxicity such as redness, erythema, oedema or eschar. There was no significant change in weight of the test animals during the treatment period and there were no mortalities. Primary Irritation Index was zero for all treatments as the parameters for its determination were absent.



A rabbit with an improvised corset



an abraded skin area of a rabbit



Rabbit healing well

Fig 6: A rabbit with an improvised corset, an abraded skin area of a rabbit and a rabbit healing well

Ocular toxicity

All the extracts of the six plants did not cause toxicity to the rabbit eye. There were no signs of eye irritation such as redness, swelling, cloudiness, oedema, hemorrhage, discharge, excessive blinking, absence of light reflex and conjunctival ulceration and blindness even after the 21-day experimental period.

DISCUSSION

Root growth inhibition was dose dependent with higher concentrations exhibiting greater inhibition. Greater root inhibition was seen with the highest concentration of 1mg/ml. There was significant difference on root length across the different doses. Concerning mitotic inhibition, for acetone extracts, significant difference among the extracts existed and mitotic inhibition was dose dependent. The most active was *Azadirachta indica* at 1mg/ml at 70.36±0.68 which was similar to that of vincristine sulphate. In the hexane extracts, the most active was *A. indica* at 1mg/ml at 71.53±1.18 and was not significantly different from vincristine sulphate. The least active was *A. Digitata* at 1.42±0.68 at 0.125mg/ml. There were

significant differences among extracts and concentrations $p<0.001$.

In the aqueous extracts, the most active was *A. indica* at 1mg/ml at a value of 67.95±0.72 which was not significantly different from vincristine sulphate. The least active was *A. digitata* at concentration of 0.125mg/ml with a value of 2.66±0.76. There was a significant difference between doses whereby mitotic inhibition increased with concentration such that the higher the dose, the greater the mitotic inhibition.

Of all the plants, *Azadirachta indica* induced greater mitotic inhibition. Several chromosomal aberrations such as micronuclei, bridges, stickiness, laggards and polyploidy have been attributed to *Azadirachta indica*.[33] This study recorded that *Azadirachta indica* induced binucleate cells and ghost cells. Formation of binucleate cells is as a result of interference between chemicals and cell wall formation. [34] *Plectranthus barbatus* induced least mitotic inhibition but there were cells which had a high cytoplasm nucleus ratio including a bulging cytoplasm. Binucleate cells, ghost cells, an increase in cytoplasm nucleus ratio and bulging cytoplasm occur in malignancies. [35]

Material to be applied on human skin should be assessed for irritability and corrosion potential. The acute dermal irritation/corrosion test is used because results obtained can be extrapolated to human. [36] It is useful in determining the mode of toxicity of a substance through the skin.[16]

All plants' extracts in this study were not irritating to both the intact and abraded skins of rabbits. They were also not irritating to the eyes of the rabbits. These plants' parts are not likely to produce irritation to the human skin and eyes. Further studies need to be carried out to evaluate whether there exists any other of form irritation in these plants because the Draize test may not fully determine mild irritation on the skin and in the eyes.

CONCLUSION

This study determined that leaf extracts of *Azadirachta indica* induces mitotic inhibition, and produces binucleate and ghost cells in the *Allium cepa* root meristems. Previous studies have shown similar outcomes including micronuclei, which is an indication of mutagenic potential. These studies identified seed extracts of *Azadirachta indica* as having greater genotoxic risk than the leaf extracts. *Azadirachta indica* and *Plectranthus barbatus* induced abnormal cells and there is a possibility of inducing the same in human. The acute dermal irritation and ocular irritation conducted did not shown any toxicity attributable to these plants hence the plants are not likely to cause severe irritation on the skin or the eyes if applied or/on contact.

Declaration by Authors

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