International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Evaluation of Anti-rodent Activity of the Root Bark of Yellow Oleander *(Thevetiaperuviana)*

John K. Ngugi[1], Joseph M. Keriko[2], Patrick G. Kareru[2]

[1, 2, 3]School of Pure Sciences, Jomo Kenyatta University of Agriculture and Technology, Kenya,P. O. Box 62000-00200, Nairobi, Kenya

Abstract: ***Yellow oleander (Thevetiaperuviana) plant extracts are toxic to most vertebrates as they contain cardiac glycosides. Cardiac glycosides are composed of steroidal aglycone with a 5 membered lactone ring and acts on cardiac muscles. The purpose of this study was to investigate the antirodent activity of the root bark extract of Thevetiaperuviana and exploit the observed activity to formulate a commercial rodenticide. The root bark extract was exhaustively extracted using Water/Methanol (1:3) solvent system by cold percolation at room temperature. The extract was characterized using different Chromatographic, spectroscopic and chemical techniques. LD_{50} of the extract was determined as well as the histopathological effect on the liver. Pellets containing different concentrations of the extract were prepared and provided as feeds to adult mice (Balb C species). The mice behavior was observed on hourly basis for the first 4hrs and after every 3hrs for the next 72 hrs. The results exhibited high acute toxicity with LD_{50} of 300mg/kg upon oral administration in mice. The histological examination indicated that the extract induced several histopathological changes in the mice. This proves that the extract from Yellow oleander (Thevetiaperuviana) can be used to formulate an environmentally friendly antirodent.***

Keywords: *Thevetiaperuviana*, $LD_{50,}$ histopathology, probit analysis

1. Introduction

Yellow oleander (*Thevetiaperuviana*)is an evergreen shrub, belonging to Apocynanceae family. It is a plant known for centuries in central and southern America, especially Peru. But it is now frequently grown throughout the tropical and subtropical regions as an ornamental plant. The cardiac glycosides found in the plant are similar to the prescription drug digoxin and have been associated with serious side effects in human leading to death. The most significant of glycoside is Thevetin. The poisonous effect of Yellow oleander has been well documented for centuries. The root decoction is an instant killer (Keriko *et al*., 2003). The Yellow Oleander's toxicity has not yet been fully exploited. This study aim at exploiting the toxicity of oleander's root barks to develop an antirodent.

Damage to crops by rodents can reduce total crop yield and increase pest control costs. This ultimately reduces the production output of the agricultural sector and all other linked sectors and could potentially have significant total economic impacts (Sexton and Silberman, 2007). In Kenya a post havest loss of 20-30 % do occur within 6 months after harvest if no interventions are made. Major losses are from insect pests, rodents and pathogens (KALRO information Brochure series 18/ 2008).

2. Materials and Methods

2.1Sample collection and pre-treatment

The root barks of the Yellow oleander were collected from Jomo Kenyatta University of Agriculture and Technology compound. The root barks were randomly selected from mature flowering trees. The barks were peeled off and sliced into small pieces in order to increase the surface area. These root barks were air dried on the Laboratory bench under room temperature for a period of three weeks.

2.2 Extraction

The dried root barks were ground into fine powder by a mechanical mill. 350g of the powder was weighed into a 2L conical flask. A methanol/water (3:1) was used to soak the powder. This content was allowed to stand under room temperature for three days with occasional shaking. The extract was then filtered through a whatman filter paper No. 3. Afterwards, the filtrates were concentrated by using a rotavapour operated at 40^{o}C and at reduced pressure so as to obtain a solid extract. It was weighed and the percentage yield calculated (Keriko *et al*., 2003).

2.3 Characterization of the extract

2.3.1Thin layer chromatographic profile of the extract

The crude methanol extracts was spotted onto a TLC plate at about 1 cm apart. Standard glycoside was Thevetia A from SIGMA Aldrich inc. The solvent system used in elution was chloroform/methanol (8:2) and detection was by UV-VIS scanner at 254 nm and 362 nm wavelengths. The TLC plate was 5 cm×20 cm, Polygram silica gel/ UV254, 0.25 mm Silica gel with fluorescent indicator from Machinery-Nagel (MN), Germany (Maryadele and Neil, 2013).

2.3.2Ultraviolet-Visible spectroscopic profile of the extract

10mg of the extract was dissolved in a10ml volumetric flask using 70% methanol. This solution was scanned ina shidmadzuUV-3600 Plus UV-Vis-NIR Spectrophotometer between 200nm and 600nm.A standard glycoside (Thevetia A) solution containing 1mg/ml was also scanned in a similar manner. All the peaks appearing in the sample were compared/matched with those from the standard.

2.3.3 Fourier Transform-Infra Red (FT-IR) Spectroscopic profile

Fourier Transform-Infra Red **(**FT-IR) spectrum can be used for molecules much as a fingerprint can be used for humans. By comparing the infrared spectra of two substances thought

to be identical, one can establish whether they are in fact identical (Yukari *et al.*, 1995*)*.

FT-IR spectra of the extract was taken in pure methanol solution and recorded on a PerkinElmer Spectrum 400Fourier Transform (FT) spectrometer employing a PerkinElmer Universal Attenuated Total Reflection (ATR) accessory. The absorption was given in wave numbers (cm^{-1}). The spectrum was recorded after background correction in the range 4000-500$cm^{-1.}$

2.4Acute toxicity test LD50

The principle of the test is based on a stepwise procedure with the use of a minimum number of animals per step. The mice (*Balb C species*) were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions (OECD, 2001guidelines for testing of chemicals).

Each dose was prepared shortly prior to administration. The test substance was administered in a single dose by gavage using a intubation canula. The extract was tested using a stepwise procedure, each step using three animals of a single sex (females). Females mice used were nulliparous and non-pregnant. Each mouse, at the commencement of its dosing, was between 8 and 12 weeks old and its weight was in an interval within± 20 % of the mean weight of any previously dosed animals. The mice were group-caged by dose. Details of the test procedure are described in **AppendexA-3.**

2.5 Determination of toxic signs and mortality in mice

A total of three mice from both sexes were used for different doses. Each dose was tested in triplicate. Four cages each with three mice were used. These mice were feed with pellets containing different extract's concentrations. The pellets were made by mixing weighed quantities of wheat flour, bees honey and beef with different concentrations of the extract. The pellets were obtained by adding a binder to the mixture and pushing the paste through a syringe to obtain pellet shapes.

2.6 Data analysis

The data was subjected to probit analysis and ANONA

3. Result and Discussion

3.1Ultraviolet-Visible spectroscopy

The Yellow oleander root extract solution was scanned between 200nm and 350nm. The spectra was done on shidmadzuUV-3600 Plus UV-Vis-NIR Spectrophotometer. **(Appendix A-1).** The standard thevetia A showed a peak at 220nm. The extract had a maximum absorbance corresponding to 220nm. A UV-VIS scan is a characteristic of specific compound. It can therefore be used in profiling the Yellow oleander root bark extract.

3.2Fourier transform infrared (FT-IR)

The sample exhibited five distinct strong peaks. The first peak was observed at 3355.7 cm^{-1}. It was a very broad trough which is probably due to the OH – stretches. The OH- stretch absorption occurs in the range 3650 to 3600 cm^{-1}. The (sp^3 -CH) stretch occurred at 2941.2 cm^{-1}. The absorption frequency for (sp^3 -CH) stretch is between 3000 and 2850 cm^{-1}. The third absorption peak was found to be at 1710.7 cm^{-1}. This peak is most likely due to the carbonyl group. The carbonyl (C=O) stretch normally occurs between the frequency of 1725 and 1705cm^{-1}. The fourth absorption peak was detected at 1643.2 cm^{-1}.This peak is probably due to (C=C) stretch which do occur between 1680 to 1600 cm^{-1}. The last noticeable intense peak was at 1028.0 cm^{-1} and was probably due to (C-O-C) ether stretch which normally occurs between 1300 and 1000 cm^{-1}. This strong and intense absorption was very useful since it confirm the presence of a glycoside linkage. The FT-IR obtained was in agreement with the functional groups present in the glycoside. This means that the FT-IR can be used in the qualitative analysis of both the extract and the formulated antirodent. **(AppendexA-2)**

3.3 Determination of LD_{50}

The extract exhibited high acute toxicity with LD_{50} cut-off of 300mg/kg body weight.

3.4 Determination of toxic signs and mortality in mice

Table 1: The mice mortality for different formulated antirodent

CAGES	Weight of extract(g)	Weight of wheat flour(g)	Weight of honey(g)	Weight of beef (g)	No. of mice used	No. of dead mice after 72hrs
CAGEA (2.63%Extract)	0.4	5	5	5	9	1 (11%)
CAGEB (5.26% Extract)	0.8	5	5	5	9	5 (56%)
CAGEC (6.25% Extract)	1.0	5	5	5	9	7 (78%)
CAGED(control)	None	5	5	5	9	none

3.4.1 The Postmortem report of the mice

The postmortem of the control and the test mice was done. **Plate 1**is a photo of the control and the test liver. The test liver was smaller in size when compared to the control. It had a lot of blood which meant some veins had raptured.

Plate 2is the photo of the small intestine. The small intestine for the test had blood all over their surface. There were swellings observed on the epithelium lining while the control was normal

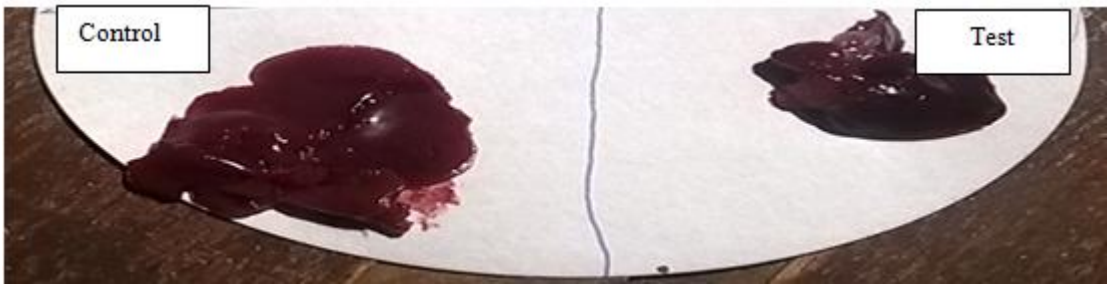


Plate 1: Effect of extract to the liver

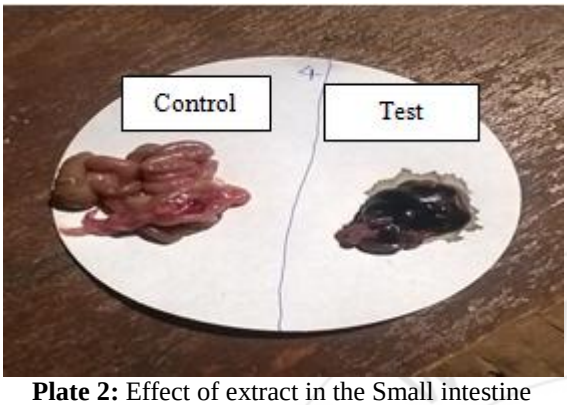


Plate 2: Effect of extract in the Small intestine

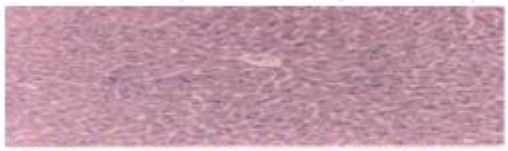


Plate 3: A sectional view of control liver

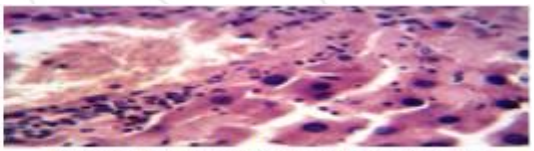


Plate 4: A sectional view of treated liver

3.4.2 Histopathology of the liver

The liver was fixed in 10% formalin, dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin. Sections were cut out at six micrometers thickness, stained with hematoxylin and eosin stain. The section was examined using a light microscope. The liver from the control was similarly examined. **Plate 3** shows a sectional view of control while **Plate 4** a section view of the treated liver.

The histopathological examination indicated that the extract induced many changes in the mice's liver. The mice developed necrosis in some hepatocytes after eating the extract. The mice also had edema and the septa between lobes were damaged. This was in agreement with other literature which had showed that glycosides cause severe damage to the liver (Sadiyha, 2013). Histopathological studies of the control liver showed normal histology.

4. Conclusion

The cardiac glycosides were effectively extracted as confirmed by FT-IR. The Yellow oleander root bark extracts exhibited high acute toxicity with LD_{50} of 300 mg/kg upon oral administration in mice (*Balb C species*). The histopathological examination indicated that the tested extract induced several physical and histopathological changes in the mice such as necrosis associated with inflammatory cell infiltration in small intestine and damage in septa between lobes of the liver. The Yellow oleander's root bark extract can be utilized as a natural alternative antirodent in agriculture production.

5. Acknowledgment

The authors are thankful to the Department of Public Health, College of Agriculture and Veterinary Service at Kabete for their assistance in various technical aspects especially in determination of LD_{50} and histology. I also thank and JKUAT's Yellow oleander project for partial funding.

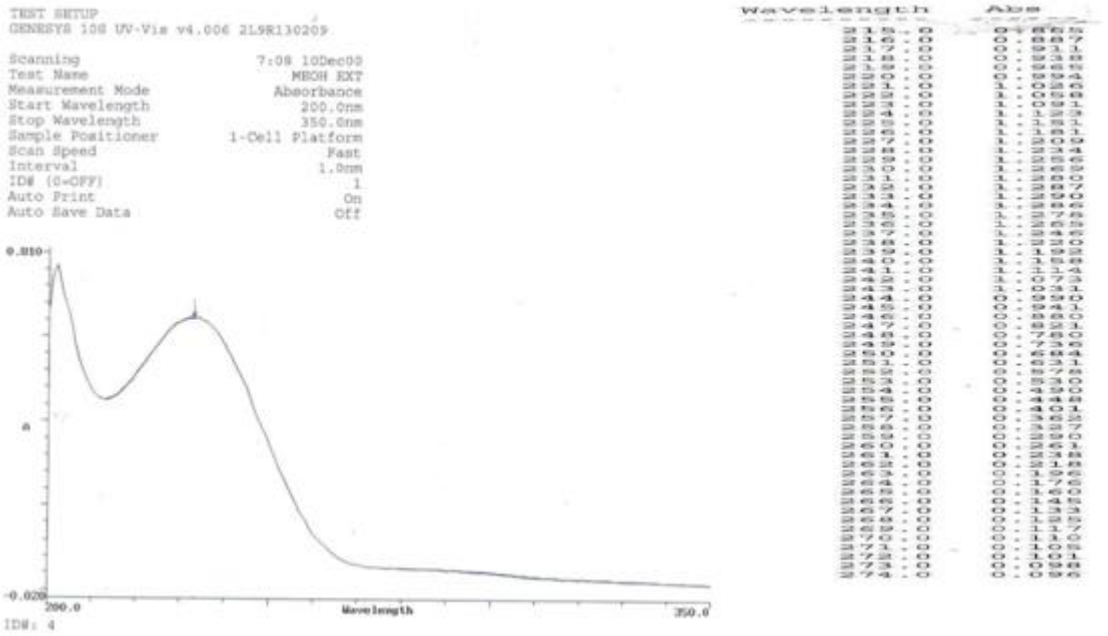
References

[1] **KALRO information Brochure series** 18 /2008
[2] **Keriko, J. M.; Gachanja, A. N., Shitanda, D. and Odhiambo, P. O. (2003).**Extraction and Utilization of yellow oleander *(ThevetiaPeruviana*) toxicity. *Journal of Americancardiol**69:*** 108-110
[3] **Lipnick, R. L.; Cotruvo, J. A. ; Hill, R. N.; Bruce, R. D.; Stitzel, K. A.; Walker A..; Chu I; Goddard, M.; Segal L.; Spiringer, J. A. and Myers R. C. (1995).** Comparison of up-and down, conventional LD_{50} and fixed dose acute toxicity procedures.*Journal of Food chemical Toxicology*.**33:** 223-231
[4] **Maryadele , J. O.(2013)** *The Merck index*.Royal Society of Chemistry; Fifteenth Edition. New Jersey, USA. ISBN-13: 978-1849736701
[5] **OECD 2001,guidline for testing of Chemicals**
[6] **SadiyhaYasir AL- Jubory.(2013)** Lethal Dose(LD_{50}) And Acute Toxicity, Histopathological Effect of Glycosides Extract Of *L. Inermis* leaves in mice. *Journal of Babylon university College of veterinary medicine.* **21:** 1-9
[7] **Sexton, S. E. Lei andSilberman, D (2007).** The economy of pesticides and pest control. *International Review of Environment and Resource Economics* **1:** 271-326.
[8] **Umavathi , S., Sathish, V., Thangam, Y. and R. Mathivanan(2015)** Analysis of
[9] phytochemical components and larvicidal activity of *Thevetiaperuviana*(*Pers)Merr*, against the

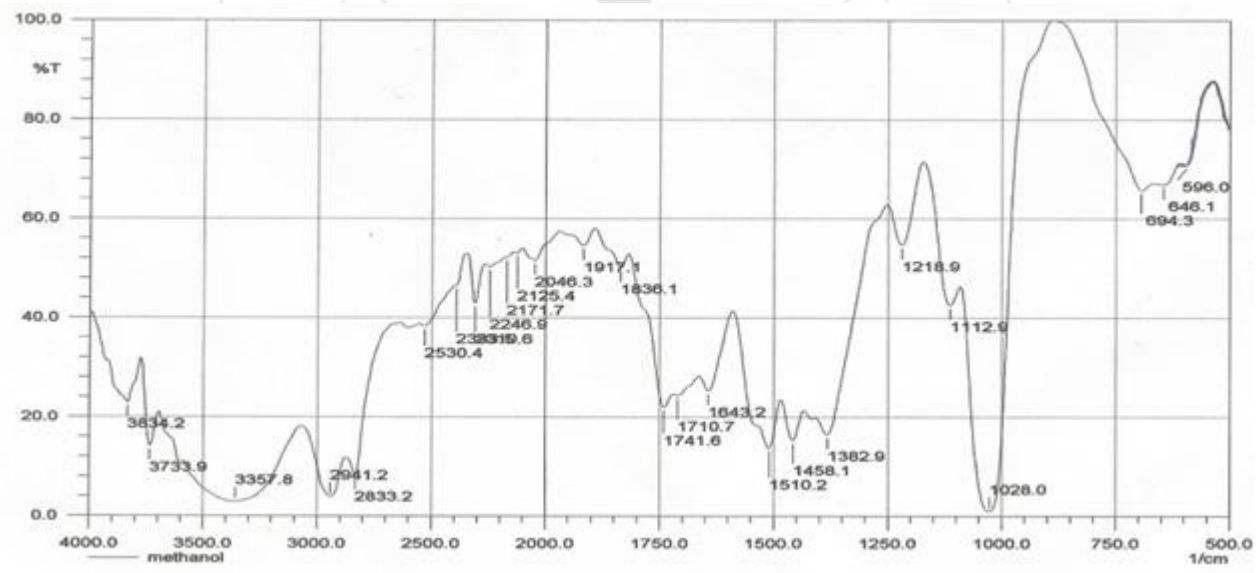
chickungunyavector.*International Journal of Current microbiology and Applied Sciences* **4:** 33-39

[10] **Yukari, I.,Youichi, F., Ikuko, N., and Mitsuru, Y. (1995).** Quantitative HPLC analysis of cardiac glycosides in *Digitalis purpurea* leaves. *Journal of Natural products***58**: (6) 897-901

Appendix 1: UV Scan for Yellow Oleander Root Extract



Appendix 2: FT-IR SCAN FOR YELLOW OLEANDER ROOT EXTRACT



Appendix A-3: PROCEDURE FOR DETERMINATION OF LD_{50}