LARVICIDAL ACTIVITY OF THE METHANOLIC EXTRACT OF *Warburgia ugandensis* SEED OIL ON *Aedes aegypti*

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ABSTRACT

Mosquito is a vector implicated with Vector-borne diseases such as malaria (Anopheles' species) dengue (Aedes species) and west Nile fever (Culex species). Kenya experiences mosquito-borne disease outbreaks with the recent ones being the dengue virus and chikungunya disease outbreak that occurred along the coast of Kenya. Medicinal plants such as *Warburgia ugandensis* that possess larvicidal activity, have major importance in the control of mosquito. This study investigated the larvicidal activity and phytochemical screening of the methanolic extract from the seed's oil of *Warburgia ugandensis.* Quasi-experimental research design was used as the study design. The plant was identified and authenticated by a taxonomist. Mature fruits of *Warburgia ugandensis* were obtained from the school of pharmacy's botanical garden and seeds separated and washed. Seeds were air dried, and crushed. Half of the GSP was used to extract seed oil while the other half was subjected to various phytochemical screening. Larvicidal activity of the plant extracts was evaluated according to the World Health Organization guidelines for laboratory and field testing of mosquito larvicides. The activity of the methanol plant extract was evaluated at 25, 50, 100, and 125 ppm to determine the LC50 value. Permethrin was used as a positive control. About 8.06% of the seed oil was extracted. The seed oil and the leaves had a characteristic odour, a bitter taste and pale yellow in color. Alkaloids, fixed oils, tannins and phyto-sterols were abundantly present in the seed. Proteins and carbohydrates were moderately present while saponins were slightly present. Bioassays on mosquito larvae indicated larvicidal activity of methanolic seed oil extract of *Warburgia ugandensis* against *Aedes aegypti* at concentrations of 25, 50, 100, and 200 ppm. Seed oil of *Warbugia ugandensis* contains phytochemicals that possesses larvicidal activity against *Aedes aegypti* mosquito species.

Keywords: Larvicidal Activity, Lethal Concentration, Lethal Dose, Warburgia Ugandensis.

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I. INTRODUCTION

Vector borne diseases account for upto 17% of the estimated global burden of all infectious diseases. Kenya being a tropical country is a known endemic zone for *Culicidae* vectors which transmit pathogens causing yellow fever, chikungunya, Zika infection and dengue fever (Karungu et al., 2019). Infection outbreaks have greatly impacted the country's economy, as resources set aside for developmental projects, are redirected and used to curb the cases of hospitalization and control such outbreaks. Occurrence of these outbreaks have been linked to the recent augmented changes affecting the environment that have led to a change in population of the infected-mosquito species within global and local boarders (Semenza, 2017).

Aedes aegypti is an arthropod vector known to transmit chikungunya, dengue fever and yellow fever. It is widely distributed in the sub-tropic and tropical zones. About two-third of the world's population, live in areas infested with dengue vectors, mainly the *Aedes aegypti* (Rogers et al., 2006). During the past decade, chikungunya, has re-emerged in Indian Ocean Islands and Africa, where they cause large outbreaks of human disease (Njenga et al., 2008). The Kenyan ministry of health (MoH), reported 453 cases including 32 laboratory-confirmed cases and 421 suspected cases from mid-December 2017 through 3 February 2018, of

chikungunya outbreak from Mombasa County (WHO, 2018). In December 2017, there were four positive cases of chikungunya and four positive cases of dengue fever determined at the Kenya Medical Research Institute (KEMRI) arbovirus laboratory in Nairobi (WHO,2018).

The main propagating factors of such outbreaks are inadequate vector control mechanisms and large mosquito breeding sites located in affected areas. The numerous open dumping sites, stagnant water, inadequate drainage system and overcrowding, creates a suitable environment for mosquitoes to breed (Castro et al., 2010). Reducing the number of artificial and natural water-filled container habitats that support breeding of mosquitoes is one of the methods employed in control and prevention. In addition, employment of larvicide technique can be used to control mosquitos. Whenever an outbreak occurs, use of synthetic insecticides is considered an effective way in eradicating flying mosquitoes (Centers for Disease Control and Prevention (CDC), 2019). where it is based on the use of synthetic insecticides, which have proven to be effective

However, increasing numbers of mosquito breeding places in urban agglomeration and emerging resistance of mosquitos to current commercial insecticides such as organo-phosphates, biological insecticides, organo-chloride, pyrethroids and carbamates have led t0 proliferation of vector-borne diseases (Wanjala & Kweka, 2018; Wanjala et al., 2015, Ondeto et al., 2017). This has triggered the research and development of environmentally indigenous, safe and biodegradable methods for vector control. Products derived from plants have received increased attention from the scientific world. Botanicals have phytochemicals that possess insecticidal activity contributing to the interruption of disease transmission at both the individual and community level (Demirak & Canpolat, 2022). Currently, more than 2000 plant species are known to contain insecticidal properties (Shaalan et al., 2005; George et al., 2014)

This study aimed to investigate the larvicidal activity of methanolic extract of *Warburgia ugandensis* seeds oil on *Aedes aegyptica.* the plant is commonly used as an alternative medicine among various communities in Kenya including the Marakwet people in managing stomachache, headache and toothache (Kipkore et al., 2014). Specifically, the study answered the questions: (1) What are the phytochemical compounds found in the *Warburgia ugandensis* seed powder? (2) What is the degree of efficacy of methanolic extract of *Warburgia ugandensis* seed oil at various concentrations against the fourth- and fifth-instar larvae of *Aedes aegyptica* mosquito? and (3) What is the diagnostic concentration required to monitor susceptibility of the *Aedes aegyptica* species of mosquito to the larvicide activity of the methanolic extract of *Warburgia ugandensis* seed oil?

II. METHODS

Quasi-experimental research was employed as a study design for this research. The plant species (*Warburgia ugandensis*) was identified and authenticated by a taxonomist/botanist and the specimen voucher stored at the School of Pharmacy (SoP) herbarium, Kabarak University. Mature fruits of *Warburgia ugandensis* were collected within the SoP's botanical garden, Kabarak University due to convenience, easily accessible. Mature seeds were separated and washed from the pulp of the fruit using running tap water in the pharmacognosy laboratory. Separated seeds were air dried at room temperature for two weeks inside the laboratory. Afterwards, the air dried seeds underwent particle size reduction using mortar and pestle to form a coarse powder. The powder was weighed and 250g of it soaked in 500ml of methanol. Methanol is a suitable solvent for extracting oil-soluble products which in this case was the seed oil. The seed oil was then extracted through maceration process. The oil was used to determine the larvicidal activity of *Warburgia ugandensis* seeds oil against specific mosquito species. The remaining portion of grounded seed powder was subjected to phytochemical screening to determine the presence of active metabolites.

Eggs of the mosquito species, *Aedes aegyptica* and *Culex pipiens*, were obtained from the entomology laboratory department of University of Nairobi. The eggs were hatched in distil water and fed fish meal. Eggs were collected on a moist filter paper. A small bowel was lined with a 3″ wide strip of filter paper. Water was added to a depth of 2.5 cm. The container was placed in a cage for adults, the egg-collecting container were left in the cage for 48 h. The bowel was removed and any excess water was drained out of the bowel. The egg paper was allowed to remain an additional 24 h in the cage, then removed, air dried for 4 days and stored by placing them in a large sealed plastic container. The eggs were hatched in deoxygenated air. Water temperature was maintained at 27°C (80°F). larvae hatched after 6-12 h and were counted using aliquot method. Approximately 70-95% of the eggs hatched in <I2 months old and occupied about 67 larva/cm2 of the water surface. A plastic tray (20 cm × 15 cm × 5 cm) was satisfactory to rear 1500 larvae. Time required to complete larval development varied from 7 to 23 days depending on temperature, food and density of larvae (Imam, et al. 2014).

The methanol crude extracts were evaluated in a preliminary screening at concentrations of 25, 50, 100, and 200 ppm. Batches of 25 4th-instar larvae and 5th-instar larvae were transferred to small disposable test dishes, each containing 100 ml of distilled water. Afterwards, one ml of aliquots of the seed extract at concentrations ranging from 25 to 20 mg/ml was added, producing final concentrations ranging between 25 and 200 ppm. Larva were exposed to these extracts subsequently at their stages of development. Activity of the methanol extract was evaluated at 25, 50, 100, and 125 ppm to determine the LC50 value. Three replicates were set up for each concentration and tests repeated 3 times on different days. Larva were exposed in an ascending series of five concentrations according to log dose. Parallel control tests were also maintained by adding 1ml of the solvent to 100 ml of distilled water. Twenty-five early 4th instar larvae were transferred to each of the beakers. Observation for the dead or moribund larvae was carried out after 24 hrs. at 25±2 °C and 75±5 % of relative humidity (RH). Permethrin (0.1 ppm; technical grade dissolved in DMSO) was used as a positive control. It was selected because it is a natural compound from the chrysanthemum flower and has been reported to be among the pyrethroids with best activity against mosquito larvae. Dimethylsulfoxide was used as solvent control (Mukandiwa et al., 2015).

Seven qualitative tests were done to screen for phytochemicals present in the grounded seed powder (GSP). (1) 0.5g of GSP was weighed, dissolved in 5ml of dilute HCL and then filtered using Whatman filter paper no. 1. Filtrate was used to check for presence of alkaloids through Mayer's, Wagner's, Hager's and Draggendoff tests. (2) 1g of GSP was dissolved in 10ml distilled water and analysed for presence of saponins through Foam and Haemolysis tests. (3) 1g of GSP was dissolved in 10ml distilled water and checked for presence of tannins through Ferric chloride, Gelatine, iodine, potassium dichromate and lead acetate tests. (4) 1g of GSP was dissolved in 5ml of chloroform, filtered and the filtrate examined for presences of phyto-sterols through Salkowski and Liebermann tests. (5) 1g of GSP was dissolved in 10ml distilled water, filtered and the filtrate subjected to Molisch's, and Benedict's tests for carbohydrate determination. (6) 1g of GSP was dissolved in 10ml distilled water and subjected to Million's, Xanthopoetic, Biuret and Ninhydrin tests for protein analysis. (7) Small quantity of GSP was separately pressed between two filter papers to check for presence of fixed oils. In addition, 3 drops of 0.5 N alcoholic potassium hydroxide was added to small quantity of GSP along with a drop of phenolphthalein. The mixture was heated over a water bath for 1.2 hours and resulting solution examined for presences of fixed oil and fats (Ramamurthy et al., 2017).

III. Data analysis

The percentage total yield was calculated using the formula; (Anokwuru et al., 2011)

$$Oil\ yield\ (\%) = \left(\frac{weight\ of\ oil\ extracted}{total\ weight\ of\ GSP}\right) * 100$$

The percentage of larval mortality and standard deviations was calculated for each concentration of the extracts and presented in form of tables and graphs. The lethal concentrations (LC 50 and LC 90) was determined at 95% confidence level using Probit analysis in Microsoft Excel. Abbott's formula was used in calculating the mortality in control whenever required.

IV. Ethical considerations

The initial study approval was obtained from the School of Pharmacy, Kabarak University. Ethics review clearance was sought from the Kabarak University Research Ethics Committee (REF NO. KUREC - 041021) while; a research license was obtained from the National Commission of Science, Technology & Innovation (License No. NACOSTI/P/21/14597). The study did not entail handling of any personal data. However, data that was emanated from the study was solely handled confidentially by the lead researcher. Further, sample collection of the plant material (only because a part of the plant was to be collected), breeding of mosquitoes and the experimenting on the mosquitoes, posed no harm to the environment.

V. RESULTS

Extraction Yields

Table 1:

Data on Plant Species Used, Total Weight of Seeds Grounded and the Percentage Oil Yield Extracted

Plant species	Part of plant	Plant extract	Extraction type	Weight of crude seed sample (powder)	Concentration of the seed oil	Percentage yield
Warburgia ugandensis	Seeds	Seeds oil	Methanolic	250g	20.15g/ml	8.06%

Organoleptic Characteristics

Table 2:

Organoleptic Characteristics of Warburgia ugandensis Seeds, Seed Powder and Seed Oil

Organoleptic characteristics	Seeds	Seed powder	Seed oil
Colour	Pale yellow	Pale-yellow-white	Pale yellow
Odour	Characteristic odour	Characteristic odour	Characteristic odour
Taste	Bitter	Bitter	Bitter

According to the study, *Warburgia ugandensis* seeds, seed powder and seed oil have a characteristic odor, a bitter taste and are pale yellow in color.

Phytochemical analysis

Table 3:

Phytochemical Analysis of Warburgia ugandensis Seed Powder Extract

Phytochemical tests	Seed powder	Constituents	
Alkaloids			
Mayer's	+++	Formation of a creamy colored precipitate	Presence of alkaloids
Wagner's	+++	Formation of a brown precipitate	
Hager's (picric acid)	+++	A yellow precipitate was formed	
Dragedroff's	+++	Formation of a reddish-brown precipitate	
Saponins			
Foam test	+	The foam formed did not persist	Presence of small amounts of saponins
Haemolysis test	-	No blood haemolysis occurred	Absence of saponins
Tannins			
5% ferric chloride	+	Formation of a light brown colour	Presence of tannins
Gelatine test	-	No white precipitate formed	
Iodine test	-	No transient red colour formed	Presence of tannins

Potassium dichromate test	+1	Formation of a light brown colour	Presence of tannins
10% lead acetate	+3	A white precipitate was formed	Presence of tannins
Phyto-sterols (Terpenoids and steroids)			
Salkowski test	+++	The upper layer turned bluish-red	Presence of terpenoids and steroids
Liebermann test	+++	Formation of a green-blue colour	Presence of terpenoids and steroids
Test for fixed oils and fat			
Spot test	+++	Presence of oily spot	Presence of fixed oils
Carbohydrate test			
Molisch test	+++	Purple ring appeared at the interface of the extract	Presence of carbohydrates
Benedict's test	++	The blue colour of Benedict's solution turned green	
Proteins			
Millon's test	++	White precipitate formed on addition of Million's reagent	Presence of proteins
Xanthopoetic test	+	The test solution turned yellow	Presence of proteins
Biuret test	++	A blue precipitate was formed	Presence of proteins
Ninhydrin test	-	No blue colour was formed	Absence of proteins

(-) absence
(+) presence
The number of (+) shows comparative amounts depending on degree of intensity on the test tube.
The phytochemical analysis conducted on *Warburgia ugandensis* seed powder indicated the presence of active metabolites in varying intensities. Result shows high level of alkaloids, phytosterols and tannins, and moderate amounts of carbohydrates.

Larvicidal bioassay

Table 4:

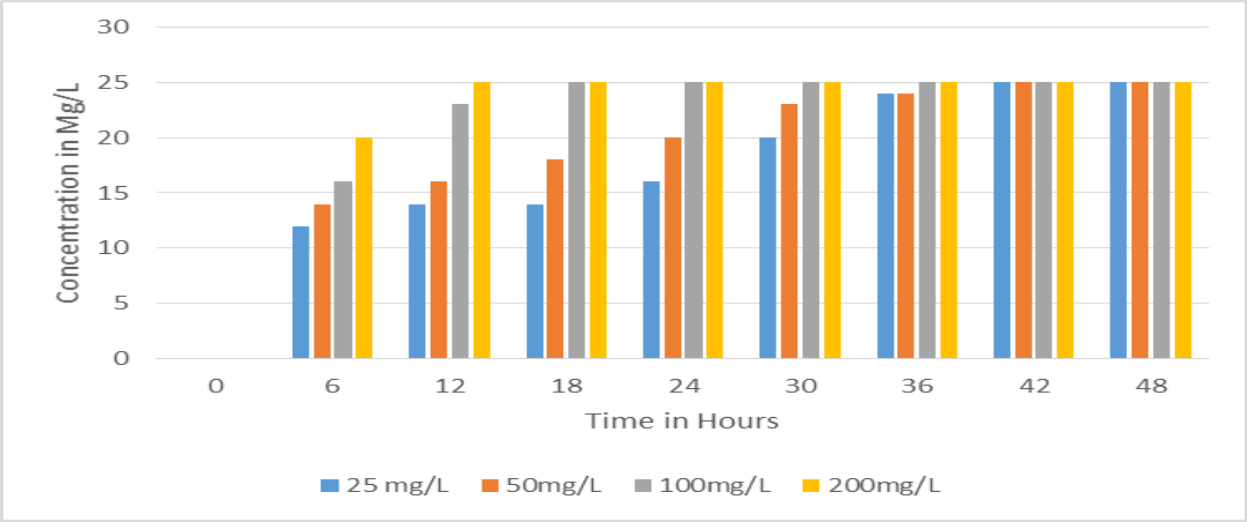
Average Number of Larvae Dead According to Different Concentrations in Relation to Time in Hours

		Time in Hours								
		0	6	12	18	24	30	36	42	48
Concentrations	25 mg/L	0	12	14	14	16	20	24	25	25
	50mg/L	0	14	16	18	20	23	24	25	25
	100mg/L	0	16	23	25	25	25	25	25	25
	200mg/L	0	20	25	25	25	25	25	25	25

The methanolic seed oil extract of *Warburgia ugandensis* indicated larvicidal activity against *Aedes aegypti* at concentrations of 25, 50, 100, and 200 ppm. Orange indicates range of LC50 while green indicates range of LC90

Figure 1:

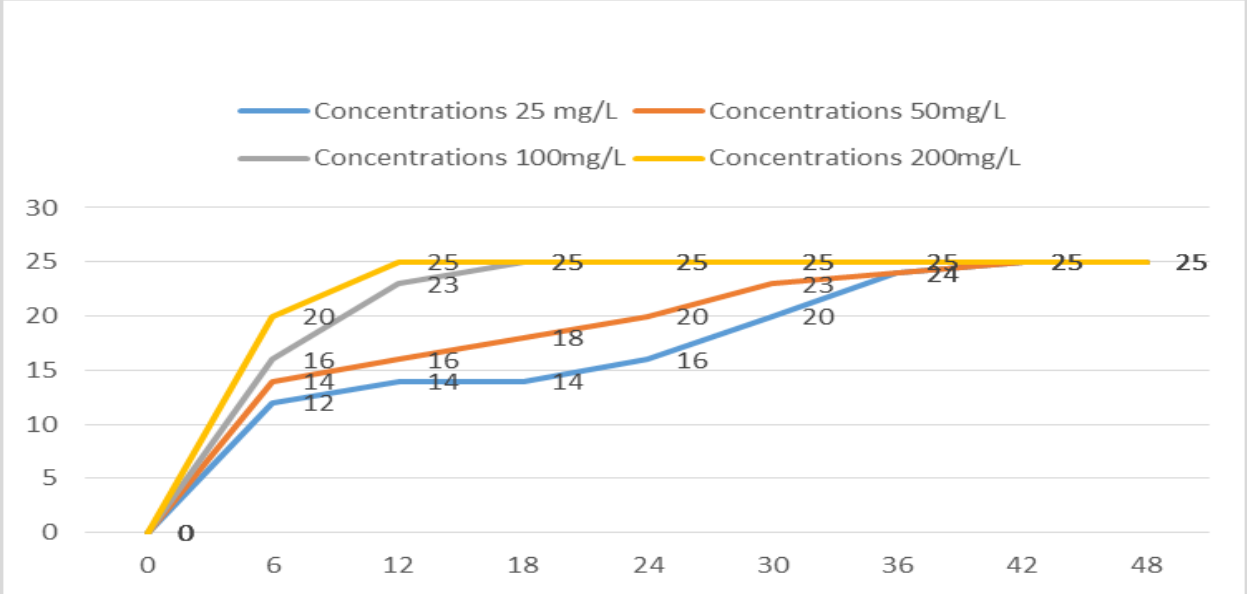
Graph on Efficacy of Methanolic Extract at Different Concentration Within Hours



The graph indicates that at the range of thirty-two to forty-eight hours, twenty-five (entire mosquito larvae population) mosquito larvae were dead at all known concentrations of 25mg/dl, 50mg/dl, 100mg/dl and 200mg/dl. At 100mg/dl and 200mg/dl concentration, the entire mosquito larvae population died after twelve hours. About half of the mosquito larvae population died after six hours of exposure to the plant extract at concentrations of 100mg/dl and 200mg/dl. Hence the higher the plant extract concentration, the higher the number of dead mosquito larvae within a few hours.

Figure 2:

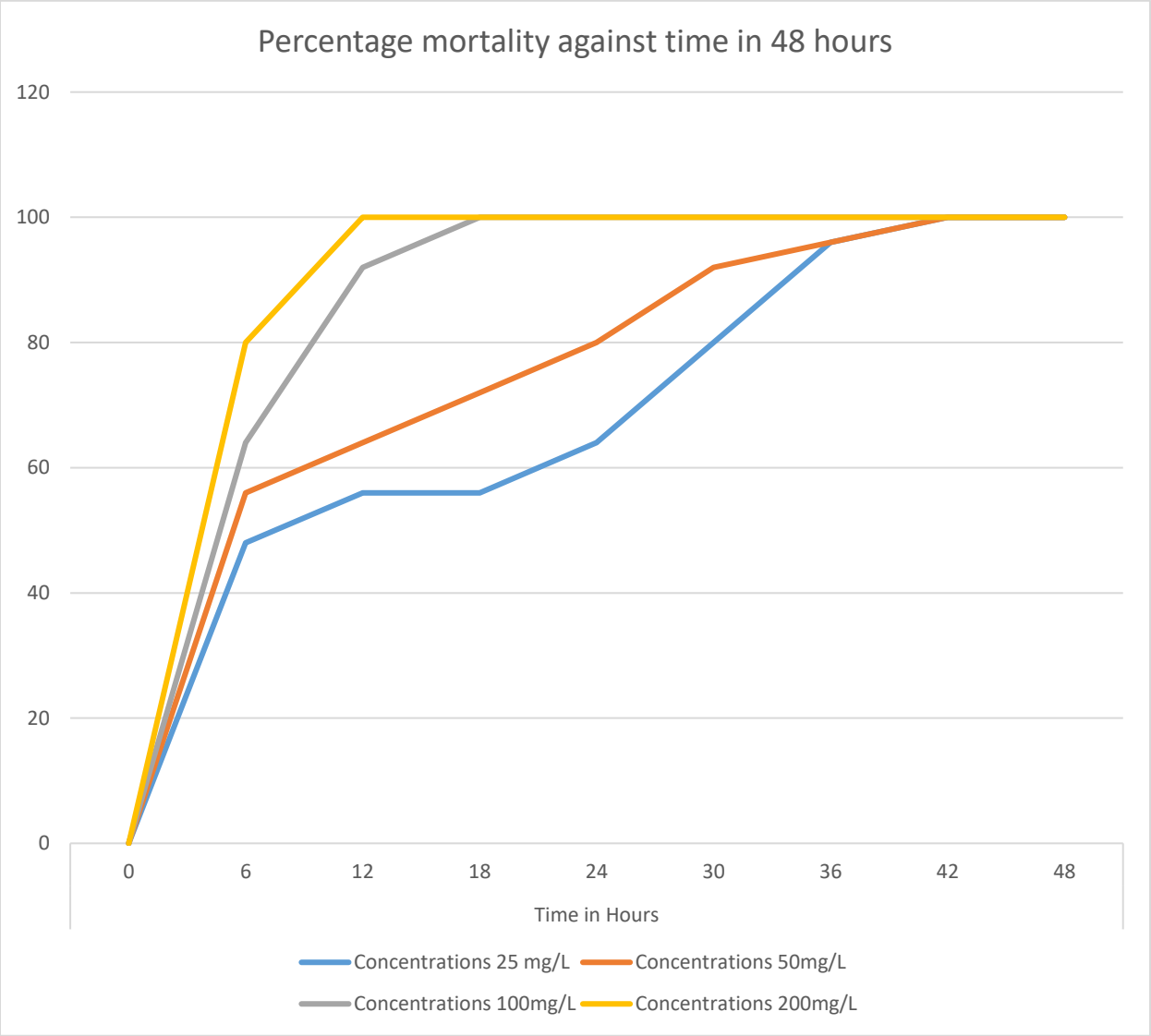
Graph on Concentration of Warburgia ugandensis in Mg/L against Time in hours (plant Extract Concentration vs Time in hours)



An increase in plant extract concentration there's an increase in the number of dead mosquito larvae as indicated with the different concentrations. The more the plant extract concentration, the more the number of dead mosquito larvae within a few hours i.e. From 12 hours to 24 hours is when majority (about half of the mosquito population) of the mosquito larvae indicated no signs of movement when pricked with a sterile needle, hence pronounced dead.

Figure 3:

Graph on percentage mortality against time in hours



VI. DISCUSSION

The results of the larvicidal bioassay utilizing different concentrations of the plant extract against *Aedes aegypti* (Table 4) indicates significant larvicidal activity with seed oil methanolic extract. The biological activity of *Warburgia ugandensis* seed oil, plant extract may be attributed to the various phytochemical compounds such as alkaloids, Terpenoids, flavonoids and phenolics. These phytochemical compounds may independently or jointly contribute to produce toxic activity against mosquito species at the larval stage.

The environmental safety of an insecticide is of utmost importance while using against vectors and pests. An insecticide does not need to cause high mortality on the target organisms in order to be considered acceptable (Kabaru & Gichia, 2009). Resistance to insecticides dates back to the commencement of using chemicals. DDT was at first introduced for mosquito control in the year 1946 and in one year's time, the first case of DDT resistance appeared in *Ae. sollicitans* and *Ae. tritaeniorhynchus* (Van den Berg, 2009). More than 500

species of arthropods are reported to be resistant to various insecticides (Mota-Sanchez & Bills, 2002). Hence, phyto-chemicals may be used as suitable alternatives to synthetic insecticides in the future, since they are inexpensive, readily available and relatively safe.

The screening of locally available medicinal plants for mosquito control will stimulate local efforts to improve public health, be cost effective and reduce the dependence on expensive imported products (Ghosh et al., 2012). Hence, this study adds on to our knowledge on the effectiveness of locally available medicinal plants.

The present study indicates that, other than all the cytotoxic, anthelmintic, medicinal property, antitumoric, antimicrobial and antibacterial property, mosquito larvicidal property is also present in *Warburgia ugandensis*. Therefore, the seed oil methanolic extract of *Warburgia ugandensis* inhibits the development of larval growth, indicating hope for further characterization of the active compound a laboratory.

VII. CONCLUSION

Findings of the study indicated that seeds of *Warburgia ugandensis* have various phytochemical compounds: alkaloids, Terpenoids, fixed oils, carbohydrates among others. These phyto-products possess larvicidal activity suggesting that *Warburgia ugandensis* may be advisable to use for control of mosquito borne diseases. The species is ecofriendly and economical with larvicidal properties. Screening of *Warburgia ugandensis* indicated high larvicidal efficacy against *Aedes aegypti*. It may be considered as an effective resource for controlling mosquito larvae. Such practice will aim to reduce the drawbacks of insecticides on the environment and provide feasible alternatives for the sustainable utilization of locally available bio-resource by campestral communities.

VIII. RECOMMENDATIONS

- There is need for further studies on *Warburgia ugandensis* to chemically isolate the active ingredient.
- A proposed structure-activity relationship and mechanism of action needs to be elucidated to help justify the plants larvicidal potential.

IX. CONFLICT OF INTEREST

Authors declare no conflict of interest.

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