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Effect of *Khaya Senegalensis* Leaves Extract against Freshwater Snail (*Lanistes ovum* Peters-1845)

Usman, Musa Aliyu¹, Shinkafi, Sa'adatu Aliyu^{2*}

Department of Biological Science, Faculty of Science, Usmanu Danfodiyo Sokoto, Sokoto State, Nigeria¹,
Department of Biological Science,
Faculty of Science, Federal University Gusau, Zamfara State, Nigeria²

Author for Correspondence: saadatushinkafi@rocketmail.com/+234-

Abstract

Toxicity experiment were conducted to evaluate the molluscicidal activity of aqueous leaf extract of *Khaya senegalensis* against freshwater snail (*Lanistes ovum*) for the control of fascioliasis disease in livestock. The toxic effect of the plant leaf extract was time as well as dose-dependent. There was a significant negative correlation between LC values and exposure periods thus, with the increase in mortality. The LC₅₀ decreased from 614.28 mg/L (24 hours), 246.22 mg/L (48 hours), 163.11 mg/L (72 hours) to 100% mortality at (96 hours). However, aqueous leaf extract of *K. senegalensis* caused no mortality against *Tilapia* fish (*Tilapia zilli*) as non-target organism, it is environmentally safe, cheap, easily biodegradable and suitable for the control of snail vector of fascioliasis disease.

Key word: Fascioliasis, *Khaya senegalensis*, *Lanistes ovum*, Molluscicidal activity.

Introduction

Fascioliasis (also known as fasciolosis, distomatosis and liver rot) is a parasitic worm infection caused by the common liver fluke *Fasciola hepatica* as well as *Fasciola gigantica*. The disease is a plant-borne trematode zoonosis (Mas-coma *et al.*, 2005). Two species of freshwater harmful snails, *Radix rubiginosa* and *Radix natalensis* are the intermediate hosts of trematodes (flukes), *F. hepatica* and *F. gigantica* which caused endemic fascioliasis in cattle and other livestock in Africa (Frag, 1998). Animals become infected with *Fasciola* spp. when metacercariae are accidentally ingested with raw vegetation, the metacercariae exists in the small intestine and moves through the intestinal wall and

peritoneal cavity to the liver and mature in the biliary ducts of the liver. Eggs are passed through the bile ducts into the intestine and finally passed in faeces (Soliman, 2008). Human are infected by eating water grown plants, primarily wild grown watercress, it may also occur by drinking contaminated water with floating young *fasciola* or when using utensils washed with contaminated water. Cultivated plants do not spread the disease in the same capacity (Mas coma., 2008). Generally human infection is rare but high among animals. High rates of human infection have been found in Bolivia, Peru and Egypt, and this may be due to consumption of certain food (Mas- coma, 2008).

Fascioliasis entails the highest economic losses on cattle production as well as severely impacting public health (Raunelli *et al.*, 2009). Today fascioliasis is recognized as an emerging and re-emerging vector-borne disease with the widest latitudinal, longitudinal and altitudinal distribution known for any zoonotic diseases (Mas-coma, 2008). Fascioliasis is found in Asia, Africa and Egypt and causes outbreak in these areas. Infection is most prevalent in region with intensive sheep and cattle production (Soliman, 2008).

Fascioliasis infection begins when metacercaria of infected aquatic plant is eaten or when water containing metacercariae is drunk. Human are often infected by eating watercress or drinking watercress juice. Cattle and sheep are infected when they consume the infectious stage of the parasite from low-lying, marshy pasture (Gorgas, 2015).

Clinical symptoms of fascioliasis in animals are abdominal haemorrhage, icterus, weakness, lethargic

anaemic, low milk production, infertility, loss of weight, gradual development of bottle jaw, emaciation, eosinophilia, elevation of liver enzyme activities and death in severe cases. But the symptoms in humans are fever, abdominal pain, gastrointestinal disturbances, loss of appetite, flatulences, nausea, diarrhoea, urticaria, cough, dyspnoea, chest pain, haemoptysis, hepatomegaly, vomiting, anaemia and jaundice (Behm, 1999).

Fascioliasis can be controlled by controlling vector snails. This can be achieved by using synthetic molluscicides but because of high cost and environmental impacts. On the other hand, plant molluscicides are cheap and safe to non-target animals and easily biodegradable in their use. Nigeria is one of the world's most endemic country in term of symptomatic/asymptomatic and number of death by schistosomiasis and fascioliasis which caused serious economic problem as well as loss in productivity. Synthetic products are expensive and present difficulties regarding the application and transport and also likely to cause environmental contamination.

Khaya senegalensis plants belong to the Family: Asteraceae, Genus: *Khaya* and Specie: *Khaya senegalensis* is a medium sized tree which can grow

up to 15-30 m in height and 1 m in diameter. The bark is dark grey to grey brown, while the heart wood is brown with a pink red- pigment made up of coarse interlocking grains, its leaves are arranged in a spiral formation clustered at the end of branches, the white flowers are sweet-scented and fruits change from grey to black when ripening. It is widely distributed to most African countries and in Riparian forest and higher- rainfall savannah woodland (IUCN, 2012). It is found in Senegal, Sudan, Uganda and savannah from 140 latitude to humid forest border (rainfall: 650- 1700mm) and was introduced to Caribbean island and France (Petit, 2000). *K. senegalensis* bitter testing bark is used for a variety of medicinal purposes; it is taken against fever caused by malaria, stomach aches and headaches. It is applied externally to cure skin rashes, wounds or any abnormality (IUCN, 2006). Its ashes are used for millet conservation; the bark is often collected for bitter properties (meliacins). Bark, resin, leaves are frequently used in human medicines for example as febrifugal, tonic, sometimes abortive and for many diseases, cure and prevent livestock diseases (Petit, 2000). Extract of *K. senegalensis* (African mahogany) showed activity against freshwater snails.



Platee 1: *Khaya senegalensis*; Source Field survey

Materials and Methods

Plant material:

The plant species were collected by hand picking of leaves from their natural habitat in Wamakko Local Government Area. The plants leaves were taken to Herbarium in the Department of Biological Science of Usmanu Danfodiyo University, Sokoto, Nigeria for identification and authentication. The plants have been identified and authenticated with the voucher number; *K. senegalensis* (UDUH/ANS/0107). The plant leaves were washed thoroughly in running tap water, shed dried after being completely dried,

leaves were pounded using mortar and pestle. The plant leaves were homogenized to fine powder with the aid of sieve and stored in air tight bottles for the extraction.

Preparation of plant Extract Concentrations:

For the preparation of *K. senegalensis* leaves extract different extract concentrations were made 200mg/L, 250mg/L, 300mg/L and 350mg/L were prepared. The powder plant material was soaked in one litre of de-chlorinated tap water with occasional vigorous shaking for 12 hours and then filtered using muslin

bag cloth. Two liters of water were added and later used for the experiment.

Qualitative Phytochemical Analyses of the plants

Previously prepared powder of all the plants materials were used. The aqueous extracts of *Senna occidentalis*, *Vernonia amygdalina* and *Khaya senegalensis* were phytochemically screened for secondary metabolites such as Tannin, Saponin, Flavonoids, Alkaloids and Cardiac glycosides (Sofowora, 2008).

Test for Alkaloids: Two milliliters of each of plant was stirred with 2ml of 10% aqueous hydrochloric acid. One milliliter was treated with a few drops of Wagner's reagent and second 1ml portion was treated similarly with Mayers reagent. Turbidity or precipitation with either of these reagents is taken as evidence for the presence of alkaloids (Harbone, 1973).

Test for Cardiac glycoside: - To 1ml of extract, 2ml of 3.5% ferric chloride solution was added and allowed to stand for 1 minute. 1ml (I of conc. H_2SO_4 is carefully poured down the wall of the tube so as to form a lower layer. A reddish-brown ring, the interface indicates the presence of cardiac glycoside (Killer-killiani's test).

Test for Flavonoid: - Three milliliters aliquot of the filtrated and 1ml of 10% NaOH sodium hydroxide, if a yellow color is developed this indicate the possible presence of flavonoid compounds (Harbone, 1998).

Test for Tannins: - Ferric chloride solution 5% ferric chloride solution was added drop by drop 2-3 ml of the extract and the colored produced is noted. Condensed tannins give blue-black color (Harbone, 1998).

Test for Saponin: Five milliliters of the extract will be placed in a test tube +5ml of water was sharked strongly. The whole tube will be filled fronth that lasts for several minutes (Harbone, 1998). To 2.5ml of the extract was added 2.5ml of Fehing's solution A and B a blue-green precipitate showed the presence of saponin (El-Olemy *et al.*, 1994).

Test for Inulin: - One milligram of the extract was added in 2 ml of water and 1 ml of Fehing's reagent which contained a mixture of Fehing's solution I and II added the mixture were heated. A brick red precipitate denoted the presence of inulin (Kasola *et al.*, 2010).

Molluscicidal activity

Molluscicidal activity evaluation of plant was carried out according to the standard procedures described by (WHO, 2012). A group of 10 untreated snails were placed in an aquarium (container), and 3 litres of tap water bubbled with atmospheric air. Experiments were conducted at 25⁰C-28⁰C. In each setup, the snails were prevented from crawling out of the container by means of a fine stainless-steel mesh placed above the water surface. The test snails were challenged with various concentration of plant extract ranging from 20 to 90mg/L. After 96 hours of exposure to plant extract, the death of snails was recorded and confirmed by the absence of heartbeat and lack of reaction to irritation of the foot with a blunt wooden probe to elicit typical withdrawal movement.

Toxicity Studies

Snails were exposed for 24 hours, 48 hours, 72 hours and 96 hours at different concentrations of single or mixture of *Senna occidentalis*, *Vernonia amygdalina* and *Khaya senegalensis* extracts. The control groups of snail were kept in similar condition without any treatment. Each set of experiments, were replicated six times. The snails were neither fed nor disturbed during the exposure and recovering period. Each dose was represented by six aquaria. Snails were daily observed and dead snails were removed immediately. The mortality was recorded after every 24 hours during the observation period of 96 hours. Contraction of the snail body within the shell and no response to a needle probe were taken as evidence of death of snails. Death snails were removed to prevent the decomposition of body in experimental aquarium. Toxicity test was performed by the method of Rawi (2011). Ten snails were kept in an aquarium containing 3 liters de-chlorinated tap water. Snails were exposed for 24 hours, 48 hours, 72 hours and 96 hours at four different concentrations of tertiary treatment. Each set of experiments, was replicated six times. Mortality was recorded after every 24hrs period of exposure. When a mechanical stimulus was applied to the foot sole, the snails was observed immediately retracted in to its shell. There will be visible swelling of the cephalopodal mass. Development of haemorrhagic "blister" over the ventral surface of the foot-sole was also observed. The active dose of plant property will cause the cephalopodal mass of each snail to become severely swollen, turgid and failing to respond to mechanical stimulation with a blunt needle. Dead snails were removed to prevent the decomposition of



the body in experiment. Mucous secretion was observed over most the foot (Gehad *et al.*, 2009). The snails were dissected and hermaphrodite glands were removed and fixed for histological examination.

Histological examinations:

For histological examinations, hermaphrodite gland of treated freshwater snails were removed after the withdrawal of the experiment, fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned. The sections were stained with haematoxylin and eosin and slide were prepared and viewed with the aid of microscope for histological observation (Rawi *et al.*, 2011).

Molluscicidal Activity Test:

Toxicity experiment was carried out according to the standard procedure described in WHO guidelines (WHO, 2012). Ten experimental animals were kept in each aquarium containing 3 litres of dechlorinated tap water and exposed continuously for 96 hours to different concentration of the plant leaves powder. Mortality was recorded after every 24 hours up to 96 hours during exposure of the snails, contraction of snail body within the shell and no response against a touch was taken evidence of death or death was confirmed loss of response to touch by a thin stainless-steel needle within the shell to elicit typical withdrawal movement. Death snails were removed to prevent the decomposition of the body in the experimental aquarium. Control animals were kept in similar condition without treatment during experimental period. Snails were kept in starved condition.

Toxic effect of the molluscicides was also studied against fish (Tilapia). In this experiment, a group of 10 fishes were exposed in 6 litres of dechlorinated tap water for 24 hours LC₉₀ (at snails) for 96 hours and observed no mortality in the exposed fishes.

The effective doses (LC values), upper and lower confidence limit, slope value, 't' ratio and heterogeneity were calculated through the POLO plus computer programme of Le Ora (1978).

Results

The result of the preliminary phytochemical screening of the plants carried out *revealed* the presence of different phytochemical compounds such as tannins, flavonoids, saponin, cardiac glycosides, alkaloides and Inulin (Table 2.)

The toxicity of the plant extract was investigated and the results obtained indicated that the plant was found effective on freshwater snail, the LC₅₀ at exposure period of 96 hours was the most effective treatment amongst all other exposure periods of 24 hours, 48 hours, 72 hours and 96 hours. The toxicity was both time as well as dose-dependent. There was significant negative correlation between LC₅₀ values and exposure periods. Thus, with increase in exposure periods and LC₅₀ values decreased.

K. senegalensis toxicity on *L. ovum*, the LC₅₀ values decreased from 614.288 mg/L (24 hours), 246.22 mg/L (48 hours) and 163.11mg/L (72 hours and 96 hours was 100%) against freshwater snails in (Table 1).

Table 1: Results of Molluscicidal Activity of *Khaya senegalensis* on Freshwater Snails Exposed to Different Lethal Concentrations

Exposure periods (Hours)	Effective dose(mg/L) LC ₅₀	Limits (mg/L)		Slope value	't' ratio	'g' value	Heterogeneity Factor
		LCL	UCL				
24h	614.288	476.43	1317.78	2.14±0.60	3.53	0.30	0.24
48h	246.22	131.04	307.99	1.68±0.55	3.030	0.41	0.22
72h	163.11	96.72	204.64	2.88±0.64	4.45	0.19	0.48
96h	100% mortality	—	—	—	—	—	—

Key: - UCL = Upper Confidence Limit

LCL = Lower Confidence Limit

"t" value = Regression Test

"g" value = Potency estimation Test

There was no mortality in fish population of *Tilapia zilli* exposed to LC₉₀ against snail. The slope values given in (Table 1) were steep. The results were found to be within the 95% confidence limit of LC₅₀.

The 't' ratio was greater than 1.96 and heterogeneity factor was less than 1.0. The 'g' value was less than 0.5 at all probability level (90, 95 and 99).

Table 2: Phytochemical Analyses of *Khaya senegalensis*.

Test	<i>Khaya senegalensis</i>
Tannins	++
Saponins	++
Flavonoids	++
Cardiac glycosides	++
Alkaloids	++
Inulin	++

++=Positive: Presence of given compound, Negative: --=Absence of given compound

The preliminary phytochemical screening of *K. senegalensis* plant proved that, African mahogany (*K. senegalensis*) of family Meliaceae present

different phytochemical compound; such as flavonoids, saponin, cardiac glycosides, alkaloides and inulin (Table 2).

Plates 2A and B: Histopathological effects on tissues of treated snails

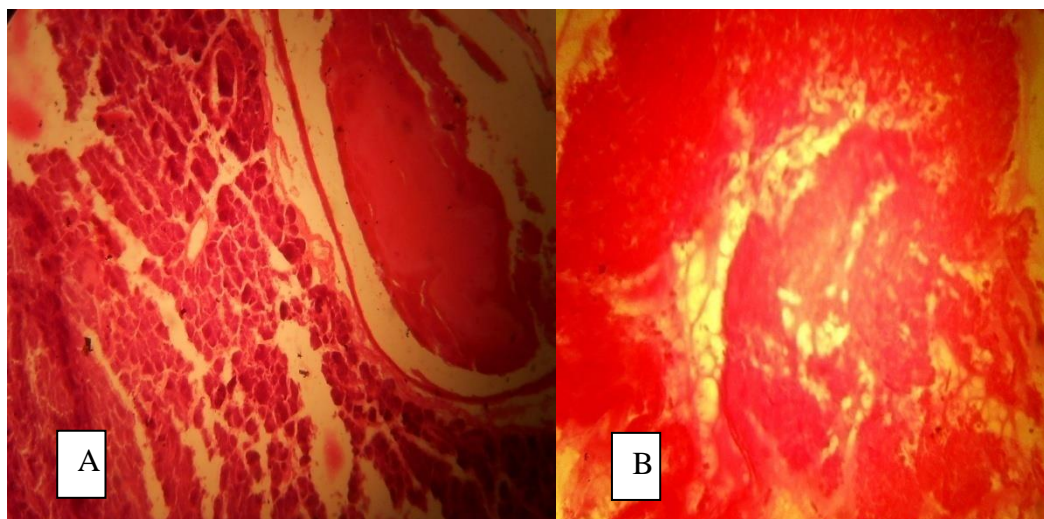


Plate 2A: Showed the hermaphrodite gland of untreated freshwater snail showing mature ova with large nuclei and surrounded by a follicular membrane, the follicular cavity is presented at different developmental stages and suggestive of intact normal tissue. (H.E X100).

Plate 2B: Photomicrograph of the hermaphrodite gland of treated snails with *K. senegalensis* (50 mg/L) after 96 hours exposure showing disappearance of follicular cavity damage of the interstitial tissue and the rupture of some organelles. (H.E X100).

Discussion

It is evident from the result that, *Senna occidentalis*, *Khaya senegalensis* and *Vernonia amygdalina* leaves extract are toxic against freshwater snails. It is clear from the result section that, high mortality in *K. senegalensis* treated snails can be attributed to the presence of high quantity of alkaloids which caused inhibition in alpha-glucosidase and reduce the glucose transport through the intestinal epithelium. It is in agreement with (Revathi *et al.*, 2015).



The phytochemical analysis of the plants used in this study showed the presence of tannins, saponin, flavonoids, cardiac glycosides, alkaloids and inulin may cause high mortality among the exposed snails in all the treatments. This finding agrees with (Singh and Singh, 2009) who reported that, in many plants the activity of compound in the exposed animal was due to the presence of saponin contents, triterpenoid and alkaloid component. It is also in agreement with a previous report (Rawi *et al.*, 2011) who revealed that saponin as the principal components that led to the high mortality, thus, high saponin in *K. senegalensis* exhibit high molluscicidal property and more cellular damage of exposed snails (Akinpelu *et al.*, 2012).

It was also observed that, there was a positive relationship between exposure period and mortality of snails. The increase in mortality with increase in exposure period could be due to several factors. These factors include exposure period/time and doses used, it may be acting separately or synergistically and caused mortality in all set up. Our finding is in agreement with finding of (Rawi *et al.*, 2011), who reported that, the uptake of plant compound used could be time dependent leading to a progressive increase in the titre of the ingredient and its effects on the snails. Thus, the exposure time is another important determination of toxic effects on exposed animals.

The individual plants are more effective than combination toward snails, histopathological damage in the hermaphrodite gland of treated snails showed severe destruction by individual plant than in combination. The effect observed are not similar but depend on the compound used. The histopathological effect on snail's gonadal organ showed slight and severe disintegration of gonadal cell, diminished in size and number. The degree of damage depends on the amount of toxicant absorbed by the snail's gonadal tissue, thus affect the transport system between the cells, this could be seen with *K. senegalensis* which caused severe damage to gonadal tissue. This finding is in agreement with (Rasheed, 2002) who reported that Lipophilic of botanical extract affect the lipid layers of the cell membrane which destroy specific permeability properties and this caused dehydration.

Conclusion

In conclusion, it could be said that all the plants *Khaya senegalensis* showed some degree of mortality in treated snails and can be used in the

control of fascioliasis diseases in the study area. The use of this plant is less expensive and may be safe to environment and readily available agent for the control of fascioliasis.

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