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Evaluation of the Molluscicidal Effects of Aqueous and Ethanol Extracts of *Carica papaya* as Potential Molluscicides against *B. pfeifferi,* Adult Snails, a Vector of Schistosomiasis

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ABSTRACT: The study was carried out to evaluation of the molluscicidal effects of aqueous and ethanol extracts of *Carica papaya* as potential molluscicides against *B. pfeifferi,* adult snails, a vector of schistosomiasis. The plant was sourced from Sokun village where it is traditionally used to treat infections. The stem bark were peeled and air dried at room temperature then crushed and sieved to standardized particles, and extracted in ethanol and distilled water. Phenols were the most abundant phytochemicals in all extracts except the seeds ethanol extracts where they were not detected. Cardiac glycosides were found in all the extracts but in trace amounts. Seeds extracts had high amounts of alkaloids while the stem lacked alkaloids. The extract of stem ethanol was the most effective, killing all the 10 snails (100%) exposed to it at the lowest and highest concentrations (50mg/l and 150mg/l). Other extracts that caused 100% mortality are the seeds ethanol extract (150 mg/l) and stem water extract (150mg/l). The maximum number of snails killed by the stem water extract was 104.65%. LD_{50} is the minimum dosage required to kill 50% of the snails. The extract of the stem ethanol had the lowest calculated LD_{50} of 2.437mg/l followed by the stem water extract (104.65mg/l). The water extract of the seed ethanol recorded the highest LD_{50} of 127.71mg/l. The maximum number of miracidia killed by the stem ethanol extract was 3 at a concentration of 10mg/l. 15mg/l and 5mg/l of the stem ethanol extract killed 1 miracidium each. The lowest LT_{50} was 70.77 minutes recorded for 10mg/l of the stem ethanol extract. LT values for 5mg/l and 10mg/l of seeds water extracts could not be computed because they did not kill miracidia. The results indicated that miracidia exhibited a high level of tolerance to all extracts from this plant. At low concentrations of 50,100 and 150µg/l no deaths were recorded for all plant extracts. When the concentrations were increased to 50, 100, and 150mg/l cercariae exhibited intolerance which was dose and time dependent. The ethanol extract of the seeds was the most lethal to cercariae. At a dosage of 100mg/l and 150mg/l all the 10 (100%) cercariae exposed to this extract were dead at 15 minutes and 45 minutes respectively. The highest concentration of seeds water extract killed a maximum of 5 cercariae. The results were subjected to Finney Probit analysis using Biostat 2009, to determine (LT_{50}) for the plant extracts. This study provides baseline information which can be used by pharmaceutical companies, researchers and the ministry of health in their quest to develop new molluscicides.

Keywords: Evaluation, Molluscicidal, Potentials, *Carica papaya*, Extracts, Schistosomiasis

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INTRODUCTION

Schistosomiasis is considered a Neglected Tropical Disease and affects more than 250 million people in tropical and sub tropical regions of the world. Sub-Saharan Africa accounts for approximately 90% of worldwide cases (WHO, 2017). Disease assessments indicate that schistosomiasis accounts for up to seventy million disability adjusted life years lost annually, considering the amount of end organ pathologies in the liver for *S. mansoni and S. japonicum* and the bladder and kidney for *S. haematobium* coupled withchronic morbidities associated with impaired child growth and development, chronic inflammation, anaemia and other nutritional deficiencies (King and Dangerfield, 2008). There is a risk of infection in fresh water of southern and sub Saharan Africa including great lakes, rivers as well as small water bodies. Transmission also occurs in South

America and some Caribbean countries (CDC, 2000). In Kenya, it is estimated that 16 million people are at risk of schistosomiasis (MOPHS, 2011). Snails do not transmit the parasite from one host to another but are an indispensable intermediate host for the development of the parasite each schistosome species uses a different snail species as an intermediate host hence availability of a suitable snail intermediate host determines the endemicity of a particular species of Schistosoma. Snails of the genus *Biomphalaria* including *B. pfeifferi* act as the intermediate hosts of *S. haematobium* in Africa. *B. pfeifferi* is the most important and most widely distributed. Reservoir hosts for *S. mansoni* such as monkeys and rodents are an important epidemiological factor of the disease in Africa and tropical America (Schmidt and Roberts, 2013).

The main drug used for treatment of all types of schistosomiasis is Praziquantel which is relatively expensive. The drug faces challenges such as not being able to kill juvenile schistosomes and risk of resistance, being the only drug suitable for mass treatment in Africa control programs (Danso-Appiah *et al.*, 2008). In the year 2015, approximately 218 million people required preventive treatment globally, Nigeria accounted for 2.5 million people (WHO, 2017). Schistosomes are blood trematodes.

Trematodes are commonly referred to as flukes. Schistosomes belong to the family Schistosomatidae which includes species that are among the most dreaded parasites of humans (Schmidt and Roberts, 2013). Schistosomes cause schistosomiasis, also known as bilharzia or snail fever (CDC, 2012). Five clinically important species cause majority of human infections (Walz *et al.*, 2015). *S. mansoni*, *S. japonicum, S. mekongi* and *S. intercalatum* mature in the hepatic sinusoids and migrate to the portal vein and its tributaries, notably the interior mesenteric vein, causing intestinal schistosomiasis. *Schistosoma haematobium* infection typically involves the bladder, lower ureters, seminal vesicles andless frequently the vas deferens, prostate and female genital system. Adult worms reside in the urinary bladder plexus causing urinary schistosomiasis. The two main species of concern in Nigeria are *S. mansoni* and *S. haematobium* (GAHI, 2010). Control of schistosomiasis has involved the use of chemical molluscicides to eliminate the snail intermediate hosts. The major challenge to use of chemicals has been their high cost and toxicity to non target organisms (Schmidt and Roberts, 2013).

The search for cheaper and environmentally friendly molluscicides from natural sources has increased over the years and plants are a major source of biologically active compounds which can give lead structures to develop new drugs (Ouedraogo *et al.*, 2016). This study investigated *C. papaya* as promising alternative sources of molluscicides and schistosomicides against *Biomphalaria* snails and *Schistosoma haematobium*.

MATERIALS AND METHODS

Study area

Lapai is located within latitude 9°03"N and longitude 6°34", it is about 18km west, its 56km East of Minna Niger state capital. Lapai covers an area of approximately 3,051km^2 whose population was estimated to be about 12, 859 (2006 census). Lapai local government area is one of the twenty-five main local governments in Niger State (Figure 1).

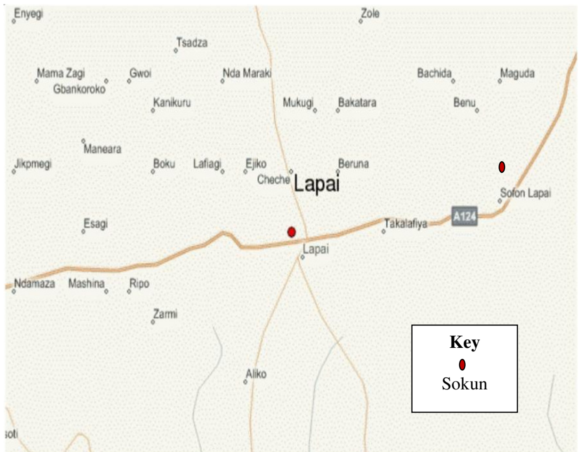

Figure 1: Map of Lapai Showing the Study Locations.

The study area is located in a tropical climate which is characterized by two distinct seasons in a year, the wet and dry seasons. The area has an annual rainfall of less than 1000mm, were rainy season which occurs between April and September with Peak Periods between October and March, a period of six months. The temperature is Lapai varies within the seasons, during the dry seasons the area record high temperature between 30°c and 36°c which last from December to April, while the rainy season experience low temperature of between 26°C and 30°C. Highest daily temperature within the season occurring at mid-day between May-July.

The natural vegetation of the study area belongs to the parkland guinea savanna vegetation characterized by a mixture of tree, shrubs and tall grasses forming a natural habitat or game and provides for a beautiful land scale and scenery, examples of common trees found in this area are: *Gmelina* spp, Locust Bean, Shea Butter trees, Isoberlina etc. several years of cultivation couples with effect of soil erosion have profoundly reduced the density of vegetation cover. The study area also has annual growth of 3.4% (national population commission, 2006).

The people are predominantly Nupe speaking with farming as the main occupation.

Study design

The study adopted is the Latin square true experimental design. Three concentrations of each plant part were used with a replicate thus each concentration formed a block of 8 samples including one negative and one positive control which were set for molluscicidal assays and similar controls for miracicidal and cercaricidal assays.

Sample analysis facilities

The research analysis was done at the Sheda Science and Technology Complex (SHESTCO) Advanced Chemistry Laboratory Abuja. Plant extractions using aquous/ethanol and phytochemical analysis were carried out at phytochemistry laboratory in (SHESTCO). Tannis, Alkaloids, Glycosides, Sterol and Triterpenes, Flavonoids, Anthraquinones, Glycosides, Saponnis, Resins.

Plant collection

The plant was identified in the department of Biology, Ibrahim Badamasi Babangida University Lapai with voucher number: IBBUL/BIO/1078 from their natural habitat in Sokun village in Lapai LGA of Niger State as herbs that are used to treat worm infections and other stomach ailments, Cough, fever, pnemonia. Small branches from the plant were then cut and labeled with a specimen voucher number then taken to the SHESTCO Advanced Chemistry Laboratory. The stem and seeds of the plant were then harvested using a knife. A sharp knife was then used to separate the seeds from the stems. The stem and seeds were washed separately in clean running water. They were then spread on newspapers under shade at room temperature (27-31°C) and left to dry (Poojory *et al*., 2015; Dhanani *et al*., 2017).

The dry plant parts were crushed using a Mekon micromealer single phase and passed through a 0.5mm mesh to standardize the particles (Micheal *et al*., 2013).

Ethanol extraction

Two kilograms of powder from the seeds and stem of *C. papaya* were placed in separate clean large bottles and two liters of 50% ethanol added until the samples were no stirring then left to soak for 72 hours. The soaked powder was filtered and the process of soaking and filtering repeated three times for each plant part (Micheal *et al*., 2013; Ganguly *et al*., 2017). Two liters of ethanol were used for each of the second and third soaking. The three filtrates of each plant part were pooled separately and clarified by filtration through Whatman filter paper then concentrated in a rotary vacuum evaporator to form a dry extract (Lymon C. Craig).

Aqueous extraction

This was done by the methods used by Adeoga *et al.* (2015) and Dhanani *et al.* (2017) with modifications. Two kilograms of the powder from the seeds and stem of each plant were placed in two separate clean large bottles and two liters of distilled water added to each bottle until the samples were completely submerged, then left to soak for 72 hours. The soaked plant parts were filtered and this process of soaking and filtering repeated three times for each plant part. Two liters of distilled water were used for each of the second and third soaking. The three extracts from each plant part were then pooled separately and freeze dried using a Lablyo plus freeze drying machine to give a dry extract. (Infitek)

Phytochemical screening

The extracts were screened qualitatively for presence of alkaloids, saponins, tannins, flavonoids, triterpenes, cardiac glycosides, glycosides, steroids, anthraquinones, phenols, flavones and resins. These were identified using characteristic colour changes by methods described by (Ayoola *et al.* (2008). Each plant extract was tested individually with specific chemical reagents according to standard procedures. Each test was qualitatively expressed as negative (-), positive (+); the intensity of the characteristic colour was expressed as ++ or +++ (Ayuk *et al*., 2015).The data was then recorded for each extract as ranked in (Table 1).

Preparation of plant extracts for molluscicidal assays

Three concentrations were prepared for each of the stem ethanol, stem water, seed ethanol and seed water extracts respectively (Table 2). By weighing the extracts from the plants (That is 4 extracts × 1 plant = 4 extracts), dissolving in distilled water, and stirring using a magnetic stirrer. The three concentrations made for each of the four extracts gave a total of 12 dissolved extracts (Table 2). Each of the dissolved extracts was then divided into two (500ml portions) for use in three replicates, giving a total of 36 experimental containers.

Collection and laboratory maintenance of *Biomphalaria pfeifferi* snails

Five hundred and fifty *Biomphalaria pfeifferi* snails were collected from water canals using scoopers (Diakite *et al.*, 2017).A layer of wet cotton wool was placed in a 5ml

Table 1: Ranks given to plant extracts according to the intensity of colour change with standard reagents.

Rank	Observation	Interpretation
-	No observed colour change	Phytochemical not detected
+	Slight Positive colour change	Trace phytochemical
++	Strong positive colour change	Phytochemical present
+++	Very strong positive colour change	Present highly present

Source: (Muhammad *et al.,* 2019).

Table 2: Concentrations of plant extracts used for molluscicidal assays

Extracts weight(g)	Amount of water added in ml	Final concentration
0.05	1000	50 mg/l
0.15	1000	150 mg/l
0.25	1000	250 mg/l

Table 3: Concentrations of crude plant extracts for Cercaricidal and Miracicidal experiments.

Extracts weight	Amount of water added in ml	Concentration
50 µg	10	5 µg/ml
100 µg	10	10 µg/ml
150 µg	10	15 µg/ml
0.05 g	1000	50 mg/l
0.10 g	1000	100 mg/l
0.15 g	1000	150 mg/l

plastic container with holes and snails placed on the cotton wool. Another layer of wet cotton wool was placed on the snails below and more snails added and covered. This was repeated until the container was full (Micheal *et al*., 2013). The container was then transported to snail laboratory. Plastic tanks of 5 liters capacity were washed using 3% hydrochloric acid and rinsed thoroughly with chlorine free water from well. Sand and gravel were collected from the snails' natural habitat and sterilized by heating at 150ºC for eleven hours, cooled then layed in tanks. Unchlorinated (snail) water from wells was then added. *B. pfeifferi* snails were washed with snail water. They were screened by exposing them to light (100 watts bulb) for five consecutive weeks. The snails were then distributed to the prepared tanks and 20daphnia added to each snail tank for aeration. The snails were housed in a temperature controlled room (27-31°C) with 12 hours light and 12 hours darkness periods (Micheal *et al*., 2013).The snails were fed on dried lettuce (FAO, 2013) and maintained at snail laboratory.

Determination of molluscicidal effects of plant extracts

Molluscicidal evaluation was done according to WHO recommended guidelines for molluscicidal tests (Gabhe *et al.,* 2016).Thirty six half liter plastic containers with perforated lids were filled with distilled water. Batches of 10 snails were pooled together in each clean 500 ml plastic containers. Dried lettuce was added to each container and left to stand for 24 hours. Distilled water was drained then the snails were challenged with50, 150 and 250 mg/l of each plant extract for 24 hours. The snails were not fed during the treatment period. Previous studies had shown that healthy snails can live for up to 5 days without feeding (Odetunji and Salawu, 2010).One negative control and one positive control were set using distilled water and niclosamide, respectively. After forty eight hours, the extracts were drained, distilled water was added and the snails were given a 24 hour recovery period. Dead snails were then identified by lack of reaction to irritation of the foot with an applicator stick and absence of heartbeat (Gehad *et al.,* 2009) when observed under a dissecting microscope. The number of dead or surviving snails was recorded for each of the treatments and controls. The fully recovered snails were kept in snail water for further use while those that died were discarded.

Preparation of plant extracts for miracicidal and cercaricidal assays

Six concentrations of plant extracts were prepared by weighing 50µg, 100µg and 150 µg then 0.05g, 0.10g and 0.15g of each plant extract and dissolving in water as shown in (Table 3).

Collection of *S. haematobium* eggs and hatching of miracidia

Miracidia for use in this experiment were obtained by hatching the eggs of *S. haematobium*.

The eggs were obtained from faeces of chronically infected baboons (*Papio anubis*) which are maintained in the laboratory. Urine were collected from chronically infected baboons in trays left under the cages for 24 hours. About 500g of urine were placed in a one liter plastic container. Half a liter of normal saline was then added and thoroughly mixed. The suspension was passed through 2 sieve meshes (size 600 and 200 µm) and the filtrate collected in a tray. The filtrate was then placed in clean 100 ml urine jars and left for 30 minutes in the dark to settle after which the supernatant was poured out. The sediment was then re-suspended in 100 ml of saline and again allowed to stand for 30 minutes (Micheal *et al*., 2013). Using a pasteur pipette, the sediment was sucked and carefully layered on a petri dish containing water to cover half of the surface then placed under artificial light (27-31ºC) for 30 minutes for miracidia to emerge based on the strong phototrophic behavior exhibited by miracidia (Jurburg *et al*., 2008).

Obtaining cercariae for cercaricidal assays

A petri dish with hatched miracidia was placed under a dissecting microscope. Five miracidia were picked from the petri dish using a drawn out glass pipette mounted with a rubber bulb. The miracidia were dispersed into each well of a 24 well microtiter culture plate (Knight *et al*., 2015). One snail was transferred to each well using a forceps and the plate covered to prevent the snails from crawling out. The plates were then left for 30 minutes for miracidia to penetrate after which snails were maintained in a 12 hours light and 12 hours darkness cycle for 3 weeks. At the fourth week, they were placed in the dark to avoid trickle shedding of cercariae (Micheal *et al*., 2013).

Shedding of cercariae from infected snails and bioassays

After 5 weeks (prepatent period), snails were removed from the dark and placed in 5 beakers containing 10 ml of snail water. The beakers were placed under light (100 watts lamp) shaded with glass and snails left for 30 minutes to release cercariae. The cercariae suspension was pooled in 100 ml beaker giving a total of 50 ml of cercariae suspension. This was then mixed well.

Assaying cercaricidal effects of the plant extracts

About 10 ml of cercariae suspension was poured into a petridish and put under a dissecting microscope. A batch of ten cercariae were picked using a drawn out pipette with a rubber bulb and placed in each well of a 24 well microtiter plate then exposed to each concentration of plant extracts prepared for cercaricidal assays at 5, 10 and 15 µg/ml followed by 50, 100 and 150mg/l. The samples were checked at minutes 5, 10, 15, 20, 30, 45 and 60 for sunken immobile cercariae. The dead cercariae were enumerated and recorded.

Analysis of data

Data on snail mortality were analyzed using Statistical Package for Social Science, (SPSS) Version23. The mean, standard errors and standard deviations of the various mortalities observed after treating snails with the various extracts of the plants at different concentrations were computed using the program. Data from each plant extract was then subjected to one way ANOVA to determine whether there were significant differences between the three dosages used. Once significant differences were identified, data was subjected to the Dunnet test to determine whether snail mortality any of the concentrations of a given plant extract was similar to the positive control (Niclosamide). The significance level used in the analysis was $P \leq 0.05$.

Molluscicidal, miracicidal and cercaricidal data was subjected to Finney probit analysis using Biostat 2009 to determine Lethal Dosage 50 (LD_{50}– Concentration of a plant extract that can kill 50 % of snails and Lethal Time 50 (LT_{50}– time taken by a specific concentration of a plant extract to kill 50 % of miracidia or cercariae).The percentage mortality and concentrations were fed into the program where mortalities were converted to probits and concentrations or time to logarithms. Logarithms were then plotted against probits to get a straight line. From the regression lines a probit value equivalent to 50 % mortality gives a logarithm which corresponds to LD_{50} or LT_{50}. The lower the LD_{50} or LT_{50}, the more effective the plant extract is in killing snails or miracidia and cercariae respectively.

RESULTS

Phytochemicals present in *Carica papaya*

Phenols were the most abundant phytochemicals in *C. papaya*(+++) in all extracts except the seeds ethanol extracts where they were not detected. Cardiac glycosides were found in all the extracts but in trace amounts (+). On average, saponins were the least detected phytochemical only trace amounts found in the seed ethanol extract. There was no uniformity in phytochemicals present in the seeds and stem; seeds extracts had high amounts of alkaloids while the stem lacked alkaloids. The stem had tannins and glycosides, while these two phytochemicals were not detected in the seeds extracts. Triterpenes were lacking in all the extracts (Table 4).

Table 4: Phytochemicals present in *Carica papaya* stem and seeds extracts.

Extract	Tannins	Cardiac glycosides	Steroids	Triter Penes	Phenols	Saponins	Glycosides	Alkaloids
Seeds ethanol	_	+	+	_	_	+	_	+++
Seed water	_	+	_	_	+++	_	_	+++
Stem ethanol	++	+	+	_	+++	_	++	_
Stem water	+++	+	_	_	+++	_	+	_

Key: Phytochemical absent + = Trace ++ = Present +++ = highly present.
Four phytochemicals (flavonoids, anthraquinones, flavones and resins) out of the twelve under investigation were not detected in plant.

Table 5: Average number of dead *B. pfeifferi* after 24 hour exposure to *Carica papaya* extracts.

Plant extract	**Number of *B. pfeifferi* dead after 24 hours (n = 10)**			
	(Mean±SE)			
Conc. mg/l	**Stems ethanol**	**Stem water**	**Seed ethanol**	**Seed water**
50	9±0.5*	2 ±0.88	1±0.5	4±1.15
150	10±0.0*	8 ±0.88	7±1.45	6±2.73
250	10 ±0*	10±0*	10±0*	7±2.73*
LD$_{50}$(mg/l)	2.437	104.65	127.71	107.53

The stem ethanolic extract was the most effective (LD$_{50}$ = 2.437).

Molluscicidal effects of *Carica papaya* plant extracts

For *Carica papaya,* thestems ethanol was the most effective, killing all the 10 snails (100%)exposed to it at the lowest and highest concentrations (50mg/l and 150mg/l). 50mg/l of this extract killed 9 out of 10 snails (90%). Other extracts that caused 100% mortality are the seeds ethanol extract (150 mg/l) and stem water extract (150 mg/l) snail mortality for these two extracts increased with an increase in concentration. The maximum number of snails killed by the stem ethanol extract was 104.65% (Table 5). The calculated Lethal Dosage 50 (LD$_{50}$) for the plant extracts gave similar results. LD$_{50}$ is the minimum dosage required to kill 50% of the snails by a given plant extract. The extract of stems ethanol had the lowest calculated LD$_{50}$ of 2.437mg/l followed by the stem water (104.65 mg/l). The water extract of the stem recorded the highest LD$_{50}$ of 127.71mg/l (Table 5). Values with * were not significantly different from Niclosamide, the positive control analysis of Variance at $p < 0.05$ significance level was used to compare snail mortality of the three concentrations of each plant extract. This revealed that there was no statistically significant difference in terms of snail mortality for the three concentrations of the seeds water extract (ANOVA df= 3; P = 0.95) and stem ethanol extract (ANOVA df = 3; P = 0.152). On the contrary, there were significant differences in terms of snail mortality among the three concentrations of *Carica papaya* seeds ethanol and stem water extracts used (ANOVA df = 3; P = 0.000). This means that the highest concentration (300mg/l) had higher snail mortality compared to 150mg/l and 50mg/l. Significant differences called for Post hoc ANOVA to compare the results with the positive control (Niclosamide). This was done using Dunnet test which showed that the three concentrations of *Carica papaya* seeds water extracts (50 mg/l, P = 1.00; 100mg/l, P = 0.095; 150 mg/l, P = 1.00) and three concentrations of *C. Papaya* stem ethanol extracts (50 mg/l, P = 0.082; 100 mg/l, P = 0.480; 150 mg/l, P = 0.230) had molluscicidal activity which were similar to that of niclosamide, the commercial molluscicide used as positive control. Other concentrations whose molluscicidal activity was not significantly different from that of Niclosamide were 300 mg/l *Carica papaya* seeds ethanol extracts (P = 1.00) and 150 mg/l *Carica papaya* stem water extracts (P = 1.00).

Miracicidal effects of the plant extracts

In the *Carica papaya* extracts, all miracidia exposed were still alive at the lapse of 1 hour except for those exposed to the three concentrations of stem ethanol extracts and 150 mg/l of seeds water extract (killed 1 miracidium). The maximum number of miracidia killed by the stem ethanol extract was 3 at a concentration of 100 mg/l. 150 mg/l and 50 mg/l of the stem ethanol extract killed 1 miracidium each (Table 6). The lowest LT$_{50}$ for *Carica papaya* was 70.77 minutes recorded for 100 mg/l of the stem ethanol extract (Table 6). LT values for 50 mg/l and 100 mg/l of seeds water extracts could not be computed because they did not kill miracidia. The results indicated that miracidia exhibited a high level of tolerance to all extracts from this plant.

Cercaricidal effects on the plant extracts

At low concentrations of 50,100 and 150µg/l no deaths were recorded for all plant extracts. When the concentrations were increased to 50, 100, and 150mg/l

Table 6: Number of dead miracidia after one hour exposure to *Carica papaya* extracts.

Extract	Concentration (mg/l)	Number of dead miracidia at the different time intervals (min)				
		30	45	60	LT_{50}(min)	SD
Seed	50	0	0	0	Not computable	-
Water	100	0	0	0	Not computable	-
	150*	0	0	1	113.08	±17.81
Seed	50	0	0	4	65.01	±6.84
Ethanol	100*	0	0	6	58.15	±5.28
	150	0	0	6	Not computed	-

Stem ethanol extract was the most lethal extract,
Stem water extract at 150mg/l was also and an effective miracidal agent
* Shows the best concentration for the each plant extract.

Table 7: Number of Cercariae dead within 60 minutes exposure to *Carica papaya* extracts

		Number of dead cercariae at various time intervals (minutes) n = 10					
Extract	Concentration (mg/l)	15	20	30	45	60	LT±SD
Seed water	50	0	0	0	0	1	113.08±17.8
	100	0	0	0	0	3	70.77±8.16
	150	0	0	0	0	5	61.07±5.94
Seed ethanol	50	0	0	0	0	0	Uncomputed
	100	0	0	0	10	10	39.57±3.37
	150*	10	10	10	10	10	6.72±5.88
Stem water	50	0	0	0	0	9	51.34±3.72
	100	0	0	0	0	10	39.57±3.37
	150*	0	0	10	10	10	6.72±5.89
Stem ethanol	50	0	0	0	0	9	51.34±3.72
	150*	0	0	0	0	10	39.57±3.37
	300*	0	0	10	10	10	6.72±5.89

*Shows the most lethal concentrations (LT_{50} 6.72 minutes).

cercariae exhibited intolerance which was dose and time dependent. The ethanol extract of the seeds was the most lethal to cercariae. At a dosage of 300mg/l and 150mg/l all the 10 (100%) cercariae exposed to this extract were dead at 15 minutes and 45 minutes respectively. Other extracts that killed 100% of cercariae were 100 mg/l and 150mg/l of the stem water extract. The highest concentration of seeds water extract killed a maximum of 5 cercariae (Table 7).The results were subjected to Finney Probit analysis using Biostat 2009, to determine Lethal Time 50 (LT_{50}) for the plant extracts. *Carica papaya* extracts did not kill cercariae before 15 minutes. The stem ethanol extract did not kill cercariae at any given dosage or time. The ethanol extract of the seeds and the water extract of the stem exhibited the highest cercaricidal activity. 150mg/l of each of these extracts had an LT_{50} of 6.72 minutes. LT_{50} for 50mg/l of the seeds ethanol extract could not be computed because this concentration did not kill any cercariae at any given time.

DISCUSSION

This study demonstrated that *Carica papaya* was very rich in saponins, glycosides and phenols but lacked Flavonoids, anthraquinones, resins and alkaloids. Phytochemicals are secondary metabolites and the differences in phytochemical composition of same plant species growing in different places can be attributed to differences in environmental factors such as geographical location which has an influence on soil type, precipitation, light intensity and temperature (Lumpkin, 2005; Kumar *et al.,* 2017). Steroids were only detected in ethanol extracts while triterpenes were only extracted by water indicating that the type of solvent used dictates the phytochemicals that are extracted. Steroids are a class of lipids with a ring system of three or more cyclohexanes and several functional groups attached. The large number of carbon-hydrogens makes steroids non polar (Ophardt, 2003) hence dissolve better in non polar solvents contrary to triterpenes which have more hydroxyl groups in their structure. In another study Hammani *et al.* (2011) demonstrated that steroids from *Solunum nigrum* were best extracted by dichloromethane which is a non polar solvent. The results differ from two other studies done which indicated that water and methanolic extracts of *Carica papaya* extracts contain abundant flavonoids and alkaloids (Sule *et al*., 2019; Audu *et al.,* 2012).

Generally, phytochemicals present in the seed of *Carica papaya* were also present in the stem almost in equal quantities. This aspect can be used for environmental conservation due to the fact that the plant quickly sprouts when the stem is cut compared to planting a seed. Herbalists who traditionally uproot the plant for medicinal purposes can be advised to consider using the stem and seeds since it contains the same phytochemicals as the roots. Phenols were the most abundant phytochemical in *Carica papaya* followed by alkaloids base on the yields from the extraction. Flavonoids, anthraquinones, resins and triterpenes were not detected contrary to a study done using methanol as a solvent where flavonoids were detected (Nthiga *et al*., 2016). Steroids were also conspicuously missing from plant extracts when water was used whereas ethanol extracts contained high concentrations of steroids. Flavonoids just like steroids are non polar with a basic structure of diphenyl propane 2 benzene rings linked by a 3 carbon chain (Udry, 2016) thus this renders them less soluble in polar solvents like water. The stem ethanol extract of *C. papaya* was the most effective in killing snails (LD_{50}2.437 mg/l). An extract with LD_{50} ranging between 0-500 mg/l indicates that the extract is highly toxic (Nguta *et al*., 2011).This implies that this extract is highly toxic to snails. The seed ethanol, stem water and stem ethanol extracts from this plant had LD_{50} of less than 500 meaning that although they were less potent compared to the seed water extract, they have good potential for use as molluscicidal agents. The high toxicity of *C. papaya* seed water extract can be attributed to abundance of alkaloids and phenols. Alkaloid components were found to be toxic to *Oncomelina hupensis* by inhibiting protein synthesis and respiratory chain oxidative phosphorylation (Wenshan *et al*., 2017). Alkaloids have been shown to kill snails in other studies (Okunji and Iwu, 1998). Srivastara (1991) also found that a quinolizine alkaloid called Virgilin from the leaves of *Calpurina aurea* killed 100 % of *Biomphalaria glabratta* at 130ppm within 48 hours. For *Vernonia amygdalina*, the stem water extract (LD_{50}, 150.92mg/l) was the best molluscicidal agent. The seed water, stem ethanol and seed ethanol though toxic, they required higher dosages. The molluscicidal ability of the plants can be attributed to presence of bio active phytochemicals such as saponins, phenols and tannins. The ability of saponins to form complexes with steroids, proteins and membrane phospholipids is responsible for a large number of biological properties especially action on cell membranes causing their destruction (Schenkel *et al.,* 2004). Guidelines from WHO recommend that for any plant to be considered as a potential molluscicide, it should be able to kill 100% of snails at 100ppm or less within 24 hours (WHO , 2020). In this study, the stem ethanol water extract of *C. papaya* killed 100% of snails at the lowest concentration of 50mg/l which is equivalent to 50ppm within the 24 hours hence it can be developed into an effective molluscicide against *Biomphalaria pfeifferi* snails. For *Carica papaya* the highest death rates were seen in 150mg/l of stem ethanol extract (LT_{50} 70.77 minutes). Miracidia exhibited tolerance to the plant extracts compared to another study done using *Entada leptostachya*, where LT_{50} for miracidia was 7.69 minutes at 80 mg/l (Micheal *et al*., 2013). Studies done using *Nigella sativa* seeds produced 100% miracidia mortality at 5mg/l after one minute of exposure (Mohamed *et al*., 2015). The most lethal extracts of *Carica papaya* were 150mg/l of stem water and 150mg/l of seed ethanol both recorded LT_{50} of 6.72 minutes. Cercaricidal effects recorded in this study are relatively higher than those obtained for *Euphobia milli* which produced 80% mortality rates after four hours exposure to 100mg/l of extracts and 73% mortality at 50mg/l (Nguta *et al*., 2011).

Conclusion

Phytochemicals present in *C. papaya* was saponins, tannins, steroids, glycosides, cardiac glycosides and phenols. For *C. papaya*, phenols were the most abundant followed by alkaloids which were present only in the seeds extracts. Extracts of *C. papaya* had abundant saponins, phenols and glycosides seeds. Water extracts of this plant also contained high concentrations of triterpenes which were not detected in *C. papaya* extracts. Flavonoids, anthraquinones, flavones and resins were lacking in the plant. The stem ethanol extract of *Carica papaya* had very strong molluscicidal properties (LD_{50} of 2.437mg/l.). *C. papaya* stem water extracts killed snails at a high dosage (LD_{50} of 104.65mg/l). Miracidia were highly tolerant to extracts from the plant. The stem water extract of *C. papaya* at 100mg/l was the most effective against miracidia (LT_{50} of 57.73 minutes).The most effective extract for *C. papaya* was stem ethanol extract at 150mg/l (LT_{50} of 70.77 minutes). Extracts from the plant killed cercariae but required at least 150mg/l to achieve 100% death rates. This concentration is relatively high hence they may not be considered candidates for cercaricidal work as standalone extracts.

Recommendations

1.There is need for considering both the stem and seeds as oppose to be whole plant for *Carica papaya* when preparing drugs since the phytochemicals found in the seeds were also found in the stem. Though, the remnants of cut stems regenerate quickly for sustainability purposes compared to propagation using seeds.
2.*Carica papaya* should be considered in development of molluscicides by pharmaceutical companies due to the high molluscicidal properties against adult snails, established in this study
3.The stem water extract of *Carica papaya* can be developed for use as a cercaricidal agent in snail habitats

where other important aquatic fauna are not present like in rice irrigation canals to protect farmers from infection because it kills cercariae fast but requires a high dosage.

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