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Evaluation of the Schistosomicidal Potentials of *Carica papaya* (Pawpaw) Extracts on the Control of Schistosomiasis: A Neglected Tropical Disease

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ABSTRACT: The study was conducted to evaluate the Schistosomicidal potential of *Carica papaya*, which is known for its broad spectrum therapeutic properties. The herb came from Sokun village, where it is traditionally used to heal diseases. The stem bark was peeled; air dried at room temperature, crushed and sieved into standardized particles, then extracted with ethanol and distilled water. Phenols were the most abundant phytochemicals in all extracts except the seeds ethanol extracts, which were undetectable. Cardiac glycosides were detected in all of the extracts, albeit in negligible levels. The seeds extracts contained a significant concentration of alkaloids, however the stem lacked them. The stem ethanol extract was the most effective, killing all ten snails (100%) at the lowest and highest doses (50mg/l and 150mg/l, respectively). Other extracts that resulted in 100% mortality include seed ethanol extract (150 mg/l) and stem water extract (150 mg/l). The stem water extract killed a maximum of 104.65% of snails. LD_{50} is the lowest dose required to kill 50% of the snails. The stem ethanol extract had the lowest calculated LD_{50}, 2.437mg/l, followed by the stem water extract (104.65mg/l). The aqueous extract of seed ethanol had the highest LD_{50} at 127.71mg/l. The stem ethanol extract killed a maximum of three miracidia at a dosage of 10mg/l. The stem ethanol extract at concentrations of 15mg/l and 5mg/l both killed one miracidium. 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 doses of 100mg/l and 150mg/l, all ten (100%) cercariae treated to this extract died within 15 minutes and 45 minutes, respectively. The highest quantity of seed water extract killed up to 5 cercariae. The findings were subjected to Finney Probit analysis with Biostat 2009 to calculate the (LT_{50}) of the plant extracts. This study provides baseline data that can be used by pharmaceutical companies, researchers, and the Ministry of Health to create new schistosomicides.

Keywords: Evaluation, schistosomicidal, potentials, *carica papaya*, extracts, schistosomiasis, neglected tropical disease

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INTRODUCTION

Schistosomes are blood trematodes which are commonly referred to as flukes (Danso-Appiah et al., 2008). 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). According to Walz *et al.* (2015), there are five clinically important species of schistsomes causing majority of human infections viz., *Schistosoma mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum.* These species matured in the hepatic sinusoids then migrate to the portal vein and its tributaries, notably the interior mesenteric vein, causing intestinal schistosomiasis. *S.haematobium* infection typically involves the bladder, lower ureters, seminal vesicles and less 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).

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 with chronic 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, 2012). 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).

The search for cheaper and environmentally friendly schistomicides 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 schistosomicides against *Biomphalaria* snails and *Schistosoma haematobium.*

MATERIALS AND METHODS

Study area

Lapai, situated at latitude 9° 03” N and longitude 6° 34” E, is located approximately 18km west of Minna, the capital of Niger State, and about 56km to the east of it. Covering an area of approximately 3,051km^{2}, Lapai is home to an estimated population of 12,859 based on the 2006 census. As one of the twenty-five main local governments in Niger State, Lapai plays a significant role in the state's administrative framework. The study area is located in a tropical climate which is characterized by two distinct seasons in a year, the wet and dry seasons (Figure 1). The area has an annual rainfall of less than 1000mm, were rainy season which occurs between April and September with a period of six months. The temperature in 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.

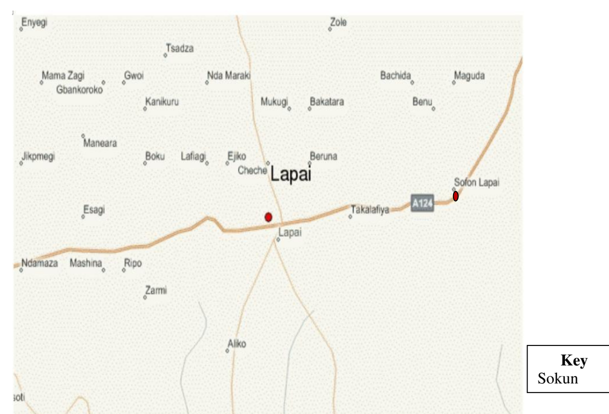

Figure 1: Map of Lapai Showing the Study Locations

Study design

Sample analysis facilities

The research analysis was done at Advanced Chemistry Laboratory, Sheda Science and Technology Complex (SHESTCO),Abuja. Plant extraction using aqueous/ethanol and phytochemical analysis were carried out at photochemistry laboratory in SHESTCO. Tanin, Alkaloid, Glycoside, Sterol and Triterpene, Flavonoid, Anthraquinone, Glycoside, Saponin, Resins.

Plant collection

The plant was collected from natural habitat in Sokun village in Lapai LGA of Niger Stateand brought to the department of Biology, Ibrahim Badamasi Babangida University Lapai and identified with a voucher number: IBBUL/BIO/1078.

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 Mekonmicromealer 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.

Aqueous extraction

This was done by the methods described 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.

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).

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)

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 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 laid in tanks. Un-chlorinated water from wells was then added. *B. pfeifferi* snails were washed with water. They were screened by exposing them to light (100 W 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 and kept at snail laboratory.

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 2).

Table 2: 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

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 (*Papioanubis*) 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 Duncan 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 $P \leq 0.05$.

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 3).

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

Table 3: Phytochemicals present in *Carica papaya* stem and seeds extracts

Extract	Tannins	Cardiac glycosides	Steroids	TriterPenes	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 4: Number of dead miracidia after one hour exposure to *Carica papaya* extracts

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	50	0	0	0	Not computable	-
Water	100	0	0	0	Not computable	-
	150*	0	0	4	65.01	±6.84
Stem	50	0	0	1	113.08	±17.81
Ethanol	100*	0	0	3	70.77	±8.16
	150	0	0	3	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 5: Number of Cercariae dead within 60 minutes exposure to *Carica papaya* extracts

Extract	Concentration (mg/l)	Number of dead cercariae at various time intervals (minutes) n = 10					
		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).

to the three concentrations of stem ethanol extracts and 150 mg/l of seeds water extract (killed 1 miracidium) (Table 4). 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. The lowest LT_{50} for *Carica papaya* was 70.77 minutes recorded for 100 mg/l of the stem ethanol extract (Table 4). 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 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 5).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 *Solunumnigrum* 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.

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 *Euphobiamilli* 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* were 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.

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. 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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