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Original Article

Biomolluscicidal Activities of Some Solvent Extracts of *Jatropha Curcas* Leaves against Vectors of Schistosomiasis

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Abstract

Jatropha curcas has emerged as a mantra amongst bioactive therapeutic plants due to its multipurpose application, bioavalability and especially certain envisaged metabolites. Notwithstanding, some problems envisaged with chemotherapeutic intervention of schistosomiasis includes; ecotoxicity, mobidity abatement and vector proliferation. The biomolluscicidal Activities of solvent extracts (chloroform, ethanol, ethyl acetate and n-hexane) of the Leaves of *J. curcas* against *Bulinus globosus* and *B. rholfsi* in a 24-h static non-renewal test was assessed. Results indicated varying degrees of mortalities, the chloroform, ethanol, ethyl acetate and n-hexane extracts against *B. globosus* had LC_{50} values of 25.00, 18.75, 41.63 and 37.40ppm respectively. Comparatively, the solvent extracts against *B. rholfsi* demonstrated a slightly higher LC_{50} values of 31.25, 25.00, 50.00 and 38.70ppm for chloroform, ethanol ethyl acetate and n-hexane extracts respectively. While, the positive control induced mortality at 1ppm in less than 24h, the snails survived the negative control within the same period. These results showed that the above named solvent leaf extracts of *J. curcas* can be applied in the integrated management of schistosomiasis.

1. Introduction

The genesis of plant therapeutic application is informed by the fact that plant produce certain metabolites as their genetic makeup, defence mechanisms and otherwise. For instance, the tannins produced by some plants induced anti-nutritional effects that results to reduced food and nutrient absorption and growth retardation [1, 2]. As established in literature, there are several metabolites identified from plants. There are over 10,000 alkaloids and 25,000 terpenes derivatives [3]. Notwithstanding, the mechanism of activities in these applied metabolites varies upon some compounding parameters [2], which includes applied solvent for extraction [4, 5], seasonal influence, location, age, individual susceptibility and environmental stresses on the plant [1, 2], or applied part of the plant such as root, stem, fruits, leaves, and seeds [6].

Jatropha is a tropical annual and temperate tolerant plant, which is usually domesticated in most settlements. The broad spectrum application of Jatropha plant is well documented [1, 5, 7, 8, 9]. This includes the antimicrobial properties [7, 10], larvicidal activities [11, 12], biofuel production [8] and molluscicidal activities activities [1, 5, 9].

Schistosomiasis is a vector-borne disease transmitted by aquatic snails belonging to several genera including; *Bullinus, Biomphalaria* and *Oncomelia*. These vectors (snails) are obligate intermediate host to flukes (parasites) of the genus *schistosoma*. The global mobidity burden of Schistosomiasis cannot be overemphasized, it ranks second to malaria amongst vector-borne diseases [13]. In Nigeria, the commonest form of schistosomiasis, being urinary schistosomiasis is becoming prevalent in rural and urban settlement [14]. Schistosomiasis vector is endemic in Asia, South America as well as the Tropical and Subtropical regions of Africa; it is contracted when persons come in contact with infected water/river that harbours the parasites [1]. The parasite are transmitted from the snails, penetrates the skin and migrates via the venous system to vital organs where they proliferates (if there is no drug intervention). Infection results from the scaring of tissues of their host. Schistosomiasis is the second most prevalent tropical and sub-tropical parasitic disease affecting 4-5% of the world population with malaria being the first [15, 13, 16]. Global statistic shows that schistotomiasis is endemic in about 70-74 countries in Africa, Caribbean, Asia, Middle East and South America [17]. The global annual incident rates is approximately 200 to 207 million [4, 17], with an incidence of symptomatic and clinical cases of 120 and 20 million respectively [17, 18]. As such multifaceted approaches tandems the control of schistosomiasis.

The challenge associated with chemotherapy of most vector-borne diseases includes; ecotoxicity [4], the risk of reinfection due to the provisional abatement of morbidity envisaged by drug intervention [12, 19], drug resistance to undeveloped forms of the parasites [19] and the unaffordability or/and unavailability of synthetic molluscicides to people in endemic area [5]. Although, as established in literatures *J. curcas* is a well-known multipurpose plant with diverse application due to certain inherent metabolites. Although the variable molluscicidal activities of several parts of the plant has been demonstrated in literature. Notwithstanding, the degree of activities is largely dependent on the applied solvent used for extraction. Sequel to these assertions, the molluscicidal activities of some leave solvent extracts of *J. curcas* is hereby investigated.

2. Materials and Methods

2.1. Plant Collection

The plant *J. curcas* was collected from Ogonokom community of Abua Local Government Area of Rivers State and transported to the Postgraduate Research Laboratory, Niger Delta University Wilberforce Island in Bayelsa State. The plant was identified using identification features as described by Wang and Ding [20].

2.2. Snail Sample

Two species of the snail *B. globosus* and *B. rholfsi* which are responsible for Schistosomiasis, especially the urinary form of schistosomiasis were collected from Kanye Dam in Kano State,

Nigeria. They were transported to the Niger Delta University's Postgraduate Laboratory.

2.3. Snail Breeding

The snails were bred in two separate aquaria (33cm x 30cm x 24cm). The aquaria were designed with stick, sand, and some stones. A 1.5V air pump was installed in order to aerate the water. The snails were identified using keys as described by Mansoorian [21]. They were bred and fed *insitu* with lettuce (commonly called salad leaves). The snails were also acclimatized at optimal laboratory conditions (33±2°C, with pH of 6.6-6.8) for several months.

2.4. Extraction Process

The leaves were shade-dried for 7 days at ambient environmental temperatures (31± 2°C). The dried leaves were further placed in hot air oven (at 30°C for 30minutes) and powdered with domestic electrical blender. Four hundred grams of the powdered leaves (400 g) were macerated in chloroform (700ml, BHD Chemical Ltd. Poole England), ethanol (700ml, Fisher Scientific international Company) ethyl-acetate (700ml, BHD Chemical Ltd. Poole England) and Hexane (700ml, BHD Chemical Ltd. Poole England) for 72h.The filtrates were extracted in a rotary evaporator (60°C), and the obtained residue was preserved at 4°C. Phytochemical screenings of the plants were carried out following standard protocols [22].

2.5 Experimental Set Up

The bioassay (i.e. dose-mortality response, was setup in triplicate) was carried out following standard procedure [23], incorporating slight modification in the method, as described by several authors [1, 6, 12, 19]. Several concentrations of the plant extracts with their respective replicates were prepared (concentrations ranging from 25-200ppm) and tested against the snails (minimum of 10 snail in each test chamber within 24 hours).

2.6 Statistical Analysis

The median lethal concentration of the snails (LC_{50}) was estimated with a dose-mortality curve (graph), using data of the average minimal lethal dose (LC_{100}). The statistical package used was the 2013 version of Microsoft excel package, with 5% error.

3. Results

The phytochemical assay of the solvent extracts (chloroform, ethanol, ethyl acetate and hexane extracts) of *J. curcas* is presented in Table 1. Generally, besides the absence of alkaloid, the result indicated the presence of phytochemicals such as phenol, tannin, flavonoid, saponins and terpenoid in all extracts of the plant. Specifically, terpenoids was predominantly present amongst all solvent extracts. Some extracts indicate moderate level of phytochemicals while the chloroform and ethanolic extracts of *J. curcas* demonstrated higher level of flavonoid and saponin as well as the conspicuous presence of phenol in the ethanolic extract.

Table 1: Phytochemical analysis of various leaf solvent extracts of the plants

Plants	Extracting Medium	Phytochemicals					
		Phenol	Alkaloid	Tannin	flavonoid	Saponin	Terpenoids
J. curcas	Chloroform	+	-	+	++	++	++
	Ethanol	++	-	+	++	++	++
	Ethyl acetate	+	-	+	+	+	++
	Hexane	+	-	+	+	+	++

++: Present in abundance; +: moderately Present; -: Absent

Table 2 shows the results of the Dose-Mortality rates as well as the range of activities of the tested solvent extracts against *B. globosus*. The result of the solvent extracts tested against *B. globosus* demonstrated varying degrees of mortalities. Notwithstanding, the ethanolic extract was the most active with a minimal average mortality rate (AMrt) of 75ppm, the chloroform extract had AMrt of 125ppm, while both ethyl acetate and hexane extracts had AMrt of 150ppm. Furthermore, the positive control was lethal at 1ppm in less than 24h, while the negative control induced no mortality throughout the bioassay.

Table 2: Results of Dose-Mortality rates activities of solvent extracts against *B. globosus*

Solvent Extracts	% MORTALITY RATES ± SD							
	200 ppm	175 ppm	150 ppm	125 ppm	100 ppm	75 ppm	50 ppm	25 ppm
Chloroform	100±0.00%	100±0.00%	100±0.00%	100±0.00%	92±0.00%	89±0.00%	79±0.00%	51±0.00%
Ethanol	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	86±0.00%	59±0.00%
Ethyl acetate	100±0.00%	100±0.00%	100±0.00%	91±0.00%	80±0.00%	71±0.00%	57±0.00%	38±0.00%
n-hexane	100±0.00%	100±0.00%	100±0.00%	94±0.00%	86±0.00%	78±0.00%	66±0.00%	40±0.00%
CONTROLS								
Positive Control	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
Negative Control	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%

Table 3 presents the results of the Dose-Mortality rates as well as the range of activities of the tested solvent extracts against *B. rholfsi*. Compared to the *B. globosus* bioassay, the result of the solvent extracts tested against *B. rholfsi* indicated lower degrees of mortalities, hence higher *AMrt* values. Notwithstanding, the ethanolic extract induced the highest activity with *AMrt* value of 100ppm, the chloroform extract had *AMrt* of 125ppm, while ethyl acetate and hexane extracts similarly had *AMrt* values of 150ppm. In addition, the positive control induced mortality at 1ppm, the snails survived the negative control.

Table 3: Results of Dose-Mortality rates activities of solvent extracts against *B. rholfsi*

Solvent Extracts	% MORTALITY RATES ± SD							
	200 ppm	175 ppm	150 ppm	125 ppm	100 ppm	75 ppm	50 ppm	25 ppm
Chloroform	100±0.00%	100±0.00%	100±0.00%	100±0.00%	89±0.00%	76±0.00%	66±0.00%	43±0.00%
Ethanol	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	81±0.00%	70±0.00%	51±0.00%
Ethyl acetate	100±0.00%	100±0.00%	100±0.00%	91±0.00%	78±0.00%	67±0.00%	54±0.00%	31±0.00%
n-hexane	100±0.00%	100±0.00%	100±0.00%	94±0.00%	83±0.00%	72±0.00%	61±0.00%	38±0.00%
CONTROLS								
Positive Control	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
Negative Control	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%

Results of the median lethal dose (LC_{50}) was statistically estimated on a dose-mortality curves (with 5% error), as presented in Figure 1 for *B. globosus*. Results of the *B. globosus* bioassay show that, the chloroform, ethanol, ethyl acetate and n-hexane extracts against *B. globosus* had LC_{50} values of 25.00, 18.75, 41.63 and 37.40ppm respectively.

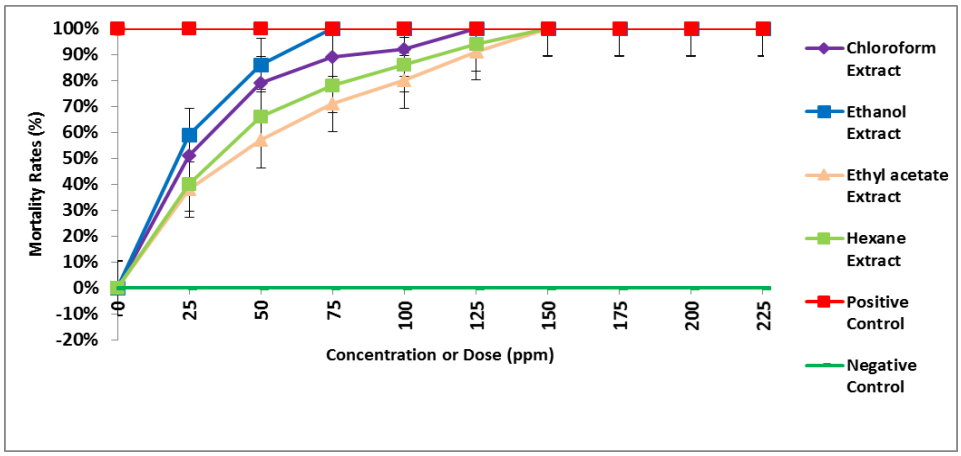

Figure 1: Dose-mortality curve for *B. globosus*

Results of *B. rholfsi* median lethal dose bioassay is presented in Figure 2. The results shows that solvent extracts *J. curcas* against *B. rholfsi* induced a slightly higher LC_{50} values of 31.25, 25.00, 50.00 and 38.70ppm for chloroform, ethanol ethyl acetate and n-hexane extracts respectively. Meanwhile, the positive control was lethal at 1ppm, while the snails survived in the negative control.

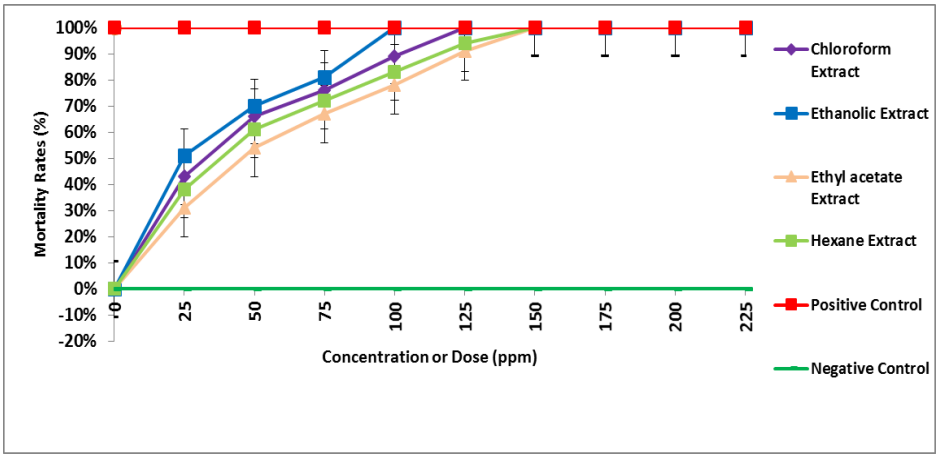

Figure 2: Dose-mortality curve for *B. rholfsi*

4. Discussion

The phytochemicals identified in the *J. curcas* our study was similarly identified for their biocidal activities by several authors in previous studies [2, 24, 25]. However, purified metabolites from the terpenes group called phobol ester is believed to be the most active metabolite in Jatropha plant. Phobol ester had been quantified (mg/g dry matter), in seeds (2-6), leaves (1.83–2.75), stems (0.78–0.99), flowers (1.39–1.83), buds (1.18–2.10), roots (0.55), wood (0.09), as well as the outer brown bark and inner green bark with 0.39 bark and 3.08 respectively [2]. Castagna et al., [26] reported that phobol esters enhance the development of protein kinase C (PKC), and it may results in the phosphorylation of different proteins as well as the reorganisation of cytoskeleton [27].

The LC_{50} values of our present research are comparable to the finding of other authors. The applied leaf solvent extracts in our current study indicated varying degree of activities between the tested species and amongst the extracts. In our previous study [1], using methanol as the solvent we reported a lower and similar LC_{50} values (1.5ppm), but different mortality time for *B. globosus* (8h) and *B. rholfsi* (7h). Notwithstanding, Al-Zanbagi et al., [28] reported the molluscicidal activities of dry and fresh, chloroform and acetone leaves extracts of *J. glauca* against *Biomphalaria pfeifferi* with LC_{50} values of 16.5 and 6.76 ppm respectively. The methanolic seed extract of *J. curcas* similarly induced LC_{50} value of 0.2ppm against *Bulinus natalensis* and *Bullinus truncates* and 25ppm against *B. pfeifferi* [9]. A previous study also shows that the root of *J. curcas* was least active with LC_{50} of 60ppm [29].

Disparities in the molluscicidal activities activities is largely dependent on compounding factors such as; the parts of the plant, locations, environmental stress, age, season of the year and genetic makeup [2], or the chemistry of the applied solvent [5], and even specie of the snail tested [1, 9]. Notwithstanding, there are diverse toxic and therapeutic metabolites found in *J. curcas* they include but not limited to; saponins, phytates, lectins, tannins, curcin, phytates and protease inhibitors [2]. However, amongst these metabolites the most potent are the diterpenes (Terpenoids), which have a derivative called phobol esters [1, 5, 9, 2].

5. Conclusion

The snails are obligate intermediate host of the schistosoma parasite, and their presence indicates the likelihood of schistosomiasis outbreak. The problems associated with chemotherapeutic intervention cannot be overemphasized. This article investigated the biomolluscicidal efficacy of some solvent extracts of *J. curcas* against vectors of schistosomiasis. The applied solvent extracts of the plant demonstrated varying degrees of mortality rates against the snail (vectors). This study corroborates several solvents can be used for the extraction and purification of *J. curcas* metabolites. We also recommend the field trial of several solvent extracts of the plant in order to actualize their appropriate dose and effects against non-targeted organisms that coexist with the snails.

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