Comparative Molluscicidal Activities of Methanolic and Crude Extracts of *Jatropha curcas* Leaves against *Biomphalaria pfeifferi*

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Research Article

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ABSTRACT

The bioassay of the molluscicidal Activities of J. curcas leaves against Biomphalaria pfeifferi was investigated in vitro, using Crude and 70% methanolic extracts in a two-phased rapid and final screening test. The rapid screening of the crude extracts, for the minimal molluscicidal concentration (LC₁₀₀), of the snails was less active (>500ppm), compared to the methanolic extracts (<500ppm). The final screening, revealed an LC₅₀ and LC₁₀₀ values of 6 and 30ppm respectively. Further investigation based on exposure, revealed a mortality within 12 hours, which meets WHO standard of less than 24 hours for a potential molluscicide. These results confirmed that J. curcas leaves can be regarded as a potential molluscicide for the control of schistosomiasis and therefore encourage the cultivation of this plant in endemic regions.

Keywords: Biomphalaria sp., Jathropha curcas, molluscicidal activities, schistosomiasis, rapid screening, final screening.


1. INTRODUCTION

Plants have evolved to produce a wide variety of toxic compounds which acts as defense mechanisms, the toxicity of these compounds to most species, ranging from microbes to humans is documented in literature (Devappa et al., 2010; Angaye, 2013; Bassey et al., 2013), these toxins, helps protect them against unwanted predators and pathogens (Ames et al., 1990). For instance, it is well documented in literature that, there are over 10,000 alkaloids and 25,000 terpenes identified from plants (Buckinghan, 1994; Cheeke, 1998; Bassey et al., 2013; Angaye, 2013), as well as phytoalexin and phytoanticipin which are precursors of plant hormone (Angaye, 2013; Bassey et al., 2013).

Furthermore, Angaye (2013) reported that the variation of plant toxin or activity strength, in terms of therapeutic metabolites may be dependent on seasonal influence and other confounding factors, which may include; age, environmental stresses on the plant, individual susceptibility, parts of the plant (root, stem, fruits, leaves, and seeds) and location. The toxicity of the plants may also depend on strength of toxin, quantity consumed, time of exposure, individual body chemistry, climate and soil, and genetic differences within the species (Devappa et al., 2010; Bassey et al., 2013; Angaye, 2013).

The fact that toxins produced by some plants may cause acute or chronic adverse effects to most organism, justify their therapeutic application as agents for combating diseases (Angaye, 2013). The plant J. curcas is adequately distributed all over the world, both in tropical and subtropical areas (Angaye, 2013), it is usually planted as “living fences” around fields and settlements (Bassey et al., 2013). Most parts of the plant are used locally in the treatment of ailments due to their antimicrobial properties (Thomas et al., 2008; Igbinosa et al., 2009). Even extracts
from the seeds have found applications as biofuel and soap (Nath and Dutta, 1997), as well as the press cake from
the seed can be used as organic manure and animal feed (Sherchan et al., 1989). It is worthy of note that
molluscicidal properties of some parts of J. curcas (especially the seeds) had been documented in literature for their
potent molluscicidal activities (Rug and Ruppel, 2000; Angaye, 2013; Bassey et al., 2013).

The World Health Organization ranks schistosomiasis as the second most endemic parasitic disease
(subsequent to malaria), affecting about 4-5% of the World population (Clerk et al., 1997; WHO, 2002, 2010; Bassey
et al., 2013). In Nigeria, there is widespread of schistosomiasis in both rural and urban settlement (Ugbomoiko et al.,
2010), which is caused by endemic parasites of the genus Schistosoma, in tropic and subtropical regions.
Schistosomiasis is contracted when persons come in contact with infected water that harbours the parasites (Bassey,
2011). The parasite penetrates the skin and migrates via the venous system to the portal veins of the intestine or the
bladder where they eventually lay eggs that scar organs and tissues (Angaye, 2013), which eventually results to
disease condition (Bassey et al., 2013).

Agboola et al. (2011) reported that chemotherapy of schistosomiasis only provides momentary abatement of
human parasites burden because of rapid re-infection rates after treatment. The drug is ineffective to immature
stages of the parasites (Bassey et al., 2013; Agboola et al., 2011), as such multifaceted approaches, like the use of
molluscicides are desirable in the control of schistosomiasis.

Notwithstanding, synthetic molluscicides are expensive and unaffordable, coupled with the fact that there is a
significant difference in dose response as it concerns different species of the intermediate host and as well as the
type of solvent used for plant extraction (Angaye, 2013). Jathropha plant have found application as molluscicides due
to certain metabolites they posses, and are evolving as a cheap and bioavailable molluscicide in schistosomiasis
control (Angaye, 2013); as such it is necessary to evaluate Comparative Molluscicidal Activities of Methanolic and
Crude Extracts of J. curcas Leaves against B. pfeifferi.

2. MATERIALS AND METHODS

2.2. Study Area

This Research on Comparative Molluscicidal Activities of J. curcas against Schistosomiasis Vector was carried out in
the Niger Delta University Postgraduate Laboratory, in the Wilberforce Island, Bayelsa State, Nigeria. Wilberforce
Island (4° 58′N; 6° 60′E) has tropical humid hot climate with two prevailing seasons, it is relatively cool in the rainy
season which lasts between March and October and the dry season is between November and February.
Wilberforce Island enjoys abundant precipitation of over 2000mm per annum and it is 45m above mean sea level.

2.3. 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.

2.4. Snail Sample

Snails belonging to the genus Biomphalaria (B. pfeifferi), which are responsible for schistosomiasis, especially the
intestinal form of schistosomiasis was collected from Kanye Dam in Kano State, Nigeria. They were transported to
the Niger Delta University’s Postgraduate Laboratory.

2.5. Snail Breeding

The snails were bred in a laboratory -acclimatized aquarium. The aquarium was designed to suit their natural habitat
with stick, sand, some stones and aerators to improve oxygen in the water. They were fed with lettuce (salad leaves)
and left for several months to acclimatize to laboratory conditions.

2.6. Extraction Process

Methanolic extraction of J. curcas leaves was carried out as described by Agboola et al. (2011) with slight
modification using 70% methanol. Triplicate 50 grams of shade-dried J. curcas leaves were pounded with mortar and
pestle and macerated in 400ml of 70% of methanol for 72 hours and thereafter the solvents was concentrated to
dryness using a rotary evaporator at 65°C leaving no trace of methanol. For the crude extract, triplicate 50g of fresh
J. curcas leaves were pounded with mortar and pestle. The juice was then squeezed and filtered with muslin cloth to separate the residue. The filtrate was then concentrated to dryness leaving no trace of liquid using rotary evaporator.

2.7. Phytochemical Screening

Prior to the bioassay, the extracts of the plant were subjected to phytochemical screening, using standard procedure (Ahirrao et al., 2011), in order to detect the phytochemical present viz., saponin, Resins, alkaloids, phenol, steroids, glycosides, tannins and flavonoids.

2.8 Experimental Set Up

Infection free snails were used for the experiment in a static non-renewal test. It was performed in line with WHO guidelines (WHO, 1965). A minimum of 10 snails per test container (beaker) were place in 500 ml of different concentrations (ppm) of the extracts, and observed every 6 hour for the number of snails crawling upwards (Guo et al., 2010). The screening was carried out in two phases, the rapid screening test and the final screening test. These screening methods are as described by Agboola et al. (2011).

2.9 Rapid Screening Test

Concentrations of 1000 ppm and 500 ppm were used to screen the snails for 100% mortality, (LC100), for both the crude and methanolic extracts on the snail. The extract which showed 100% mortality at 500ppm was then selecte d for the final screening.

2.10 Final Screening

The extract that performed at 500ppm and below (≤500ppm) was used for this screening to determine the lowest lethal concentration, LC100, at different concentrations in a descending order. One part per million Copper sulphate was used as the positive control while de-chlorinated water was used as the negative control.

2.11 Statistical Analysis

The lethal concentration that killed 50% of the snails (LC50) was determined using the SPSS version 20, while graphs were plotted using Microsoft excel with 5% error.

3. RESULTS AND DISCUSSIONS

The results of the tested plant extract, screened against B. pfeifferi (Tables 1-3 and figure 1), was carried out based on methods of determining plant efficacy, according to standards required for the screening of molluscicide (WHO, 1965) and by determining the toxicity of crude and methanolic extracts of J. curcas leaves against snails of the genus B. pfeifferi which is a vector of the intestinal form of schistosomiasis.

The phytochemical screening of the crude extract, indicated the presence of some phytochemicals like; Alkaloids, flavonoids, saponins, and tannins; which are believed to be hydrophilic (Rug and Ruppel, 2000). On the other hand, most phytochemicals indicated in the crude extract were either weakly present or absent in the methanolic extract. Notwithstanding, a hydrophobic compound called phobol esters, which belongs to the diterpenes group (Angaye, 2013), was indicated in the methanolic extract, due to the strong presence of terpenoids (Table 1).

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemicals</th>
<th>Crude Extract</th>
<th>Methanolic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sterols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Saponin</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Tannins</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Phenol</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Terpenoids</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>9.</td>
<td>Resins</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Keys: + = weakly present, ++ = strongly present, - = absent.
The crude extract performed poorly (>500ppm), during the rapid screening (table 2), which is largely attributed to its poor extraction or unavailability of some bioactive compounds (phobol ester), compared to the methanolic extract (Angaye, 2013; Bassey et al., 2013; Hirota et al., 1988) or due to hydrophilic contents in J. curcas (Rug and Ruppel 2000; Stirpe et al. 1976; Makkar et al. 1997), be that as it may, the extremely high concentration of the crude extract (>500ppm), that killed the snail may not favour its use as a molluscicide (Angaye, 2013; Bassey et al., 2013; Rug and Rupel, 2000). This result agrees with previous investigations by Angaye, (2013) and Rug and Rupel (2000), whose investigations revealed an LC_{100} values of greater 1000ppm with crude extract of J. curcas leaves and seeds respectively.

<table>
<thead>
<tr>
<th>Concentrations (ppm)</th>
<th>Mortality Rate (%)</th>
<th>Minimal Lethal dose LC_{100} (ppm)</th>
<th>Median Lethal dose LC_{50} (ppm)</th>
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<tbody>
<tr>
<td>400-450</td>
<td>100</td>
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<td>350-400</td>
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<td>50-100</td>
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<tr>
<td>0-50</td>
<td>100</td>
<td>30</td>
<td>6</td>
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<tr>
<td>Positive control</td>
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<td>0.5</td>
</tr>
<tr>
<td>Negative control</td>
<td>0</td>
<td></td>
<td>0</td>
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</tbody>
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Key * = End point (Minimal Lethal Dose i.e. LC_{100})

On the other hand, the methanolic extract of the leaves was very active to the snails with a mortality less than 10 hour (Table 3). These values were extremely more active for methanolic extract, compared to previous studies of chloroform extract of dry leaves of J. glauca which showed an LC_{50} of 16.5 ppm and LC_{90} of 46.8ppm against B. pfeifferi and the acetone extracts of fresh leaves which had LC_{50} of 6.76ppm and LC_{90} of 12.5 ppm (Al-Zanbagi et al., 2000).

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<tr>
<td>Negative control</td>
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<td>0</td>
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</tbody>
</table>

Key * = End point (Minimal Lethal Dose i.e. LC_{100})

In our investigation, we used methanolic extracts of J. curcas leaves against B. pfeifferi and obtain an LD_{50} value of 6ppm (Table 2). In previous studies, ethanol/methanol or crude extracts of the roots (El Kheir and El Tohami, 1979), leaves (Bassey et al., 2013) and the seeds (Rug and Ruppel, 2000) of same plant were screened against Bulinus truncatus and B. natalensis from Sudan and Egypt, B. rholfsi and B. globosus from Nigeria and laboratory bred B. glabrata from America; the LD_{50} values for ethanolic root extract (60ppm) was very high compared to the leaves and seeds (0.2 and 0.3ppm respectively).
Furthermore, in our study, it is worthy of note that during the screening, all snails died in less than 12 hours which meets WHO standards of less than 24 hour for an active molluscicide (figure 1). These snail used; belongs to the genus *Biomphalaria* which host parasites that causes intestinal schistosomiasis; had higher LD$_{50}$ value (6ppm) compared to previous studies of 0.2 and 0.3ppm by Bassey et al. (2013) and Rug and Ruppel (2000) respectively for snails of the genus *Bulinus* which is responsible for urinary schistosomiasis, This variation of LD50 value for different genera suggest that the lethal dose required for one genus is independent of another (Angaye, 2013; Bassey et al., 2013).

There are several phytochemicals present in *J. curcas* such as; saponins, curcin, phytates and protease inhibitors, which are well documented in literature; notwithstanding the most potent amongst them are the diterpenoids (Terpenoids), groups of compounds which have a tigliane skeleton called phobol esters (Rug and Ruppel, 2000; Devappa et al., 2010; Bassey et al., 2013; Angaye, 2013), which are only enriched in methanolic extracts (Hirota et al., 1988). Phorbol esters are direct activators of the protein kinase C (PKC) (Castagna et al., 1982), which may cause phosphorylation of different proteins and subsequent restructuring of cytoskeleton (Bershadsky et al., 1990). Makkar and Becker (2009) who quantified phobol ester in different parts of the plant, discovered that the concentration of phobol esters (mg/g dry matter) in *J. curcas* varied from (2-6) in seeds, the leaves have (1.83–2.75), stems (0.78– 0.99), flowers (1.39–1.83), buds (1.18–2.10), roots (0.55), bark (outer brown skin) (0.39), bark (inner green skin) (3.08) and wood (0.09). It is already established in literature that the seed of *J. curcas* is the most effective therapeutic agent; notwithstanding, it may not be available throughout the season, like the flower. Exploiting the sap, stem or root might cause severe injury to the plant in what seems to be a destructive sampling.

Angaye (2013) reported that the exploitation of the leaves of a particular plant; if effective at a reasonable concentration, should be a better/best-fit molluscicidal/therapeutic agent compared to other parts such as the stem, root, sap, bark or flower; because the exploitation of the leaves is bioavailable in and out of season, and will not cause irreparable harm to the plant. It is worthy of note that, the exploitation of some plants by researchers might be destructive as it concerns the ecosystem; in the case of *J. curcas* it thrives in extreme climate and marginal soil (Heller, 1996).

**CONCLUSION**

The methanolic extract of the leaves of this plant were very effective to the snails (vector) at a reasonable concentration, and could become a cost-effective component of an integrated approach to the control of schistosomiasis. Furthermore, the therapeutic value of the plant is multifaceted in nature; as such people in endemic area are therefore encouraged to farm the plant.

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