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Plant Molluscicides and their Modes of Action: a Review

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Abstract

Molluscicides are crucial in the control of shistosomiasis. A number of plant species has been shown to have molluscicidal effect on vector snail of schistosomes, Biomphalaria sp. And Bulinus sp. this ranges from those that had very strong molluscicidal activity, 0.1-10ppm, moderate to strong=50-100ppm, mild to moderate =100-200ppm, weak to mild = 200-400ppm. The molluscicidal action was attributed to the presence of bioactive substance such as alkaloids and saponins that interferes with metabolic activities in snail vectors leading to paralysis and subsequent death of the animals. Major challenges that hinder the development of plant molluscicide are shift from disease control to morbidity control using praziquantel, problem with extraction and isolation of bioactive substance and application protocols involved in the use of plant molluscicide. Although snail control has its limitation, snail control by the use of molluscicide of plant origin appears to be of economic and ecological advantage.

Keywords: *Molluscicides, Plant, Vector snail*

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Background to the Study

Mollusks are a group of soft-bodied animals that includes snails, scallops, clams, and sea slugs. The phylum mollusca including snails and slugs (gastropoda), mussels and clams (bivalvia), squids and octopuses (cephalopoda), and a few other less well-known species distributed in five other classes), is the second most diverse taxonomic group of animals in terms of numbers of described species, after the very species-rich group of arthropods (Parent, 2008). The class gastropoda contain snails and slugs which are of economic importance, they serve as source of foodand source of important luxury goods, notably pearls, mother pearl, tyrian purple dye, and sea silk (Tabugo et al., 2013). They are also detrimental to human, as pest of crops and vectors of human disease caused by trematodes, notably schistosomiasis and fascioloisis (WHO, 1983).

The vector snails are aquatic and act as an intermediate host for the development of the parasite to an infective free swimming larval stage, i.e. cercariae for schistosome or metacercarie for fasciola.Most intermediate hosts of human Schistosoma parasites belong to three genera, biomphalaria, bulinus and oncomelania while the species belonging to the genus lymnaea are of importance in the transmission of liver flukes. Lymnaea species may be either aquatic or amphibious (Mandal, 2015).

The best way to break the cycle of infection and reinfection with these parasitic diseases is by effective control of the snail intermediate host. Use of molluscicides is the most frequent public health intervention, as it prevents the transmission of many other trematodes. This can be achieved by the use of synthetic and vegetable agents in the bid to kill the intermediate host in water (Oniya et al., 2013). Synthetic molluscicides such as copper sulphate are fairly toxic to aquatic animals, niclosamide are fairly specific and problem with modern molluscicides is that they are expensive and are harmful to the environment and non-target organisms and they accumulate in the food web (Thomas, 1995), along with increasing concern over the possibility of snail resistance to these compound (Al-Zanbagi, 2013). Plants naturally produce compounds through secondary metabolism that provide defense against herbivores this is attributed to their biocidal activityand are therefore, used in folk medicine as bactericides, fungicides, nematicide, anthelmintic and acaricides among others (Al-Daihan, 2008). Several reviews on plant molluscicides have been available during the past decades (Kloos and McCullough, 1984; Pereira de Sauza, 1995; Alzanbagi, 2013). The search for plant molluscicide is geared towards obtaining plants with molluscicidal properties which may be uncomplicated, economical and suitable technique (Kloos and McCullough, 1984), molluscicides of plant origin has been opined to be a simple, in expensive and safe alternative (Singh and Singh, 2003; Abdallah et al., 2011; Asemota et al., 2015) . However, a good molluscicide requires the following characteristics;

a. Should be toxic to the snail at low concentration.
b. Should not be toxic to mammals, that is should neither presenting acute or chronic toxicity.
c. Should not have any adverse effects if it enters the food chain.
d. It should be of low cost. (Pereira de Sauza, 1995).

Aims

A number of plant species have been shown to have molluscicidal effects on vectors snail. This review highlighted some plants with known molluscicidal properties, general mode of action and factors that affect efficacy of molluscicide.

Table 1: Plants Molluscicide and Their Lethal Concentration (LC_{50})

s/n	Name of plant	Family	English name	Specie of snail	LC_{50} in ppm	Chief active moiety	Investigator /s
1	*Euphorbia splendens*(dl &Cw)	Euphorbiaceae	Crown of thorns or Christ plant	*Biomphalaria alexandrina*	40	Milliamine L,(Schall, 1998)	Bakry, (2009a).
2	*Atriplexstylosa*(dl &Cw)	Amaranthaceae	Orache or salt bush	*Biomphalaria alexandrina*	95	Unknown	Bakry, (2009a)
3	*Guayacumofficinalis*(dl &Cw)	Zygophyllaceae	Hard back or Tree of life	*Biomphalaria alexandrina*	120	Guaifenesin	Bakry, (2009a).
4	*Calotropisprocera*(dl)	Asclepiadaceae	Sodom apple or Giant milk weed	*Bulinus truncatus*	619	Calotropin, (Onike, 2012)	Abdallah*et al.*, (2011).
5	*Nicotianatobacum*(dl)	Solanaceae	Tobacco	*Bulinus truncates*	805	Nicotyme (alkaloid)	Abdallah*et al.*, (2011).
6	*Trigonellafoenum*(s)	Papilionaceae	Fenugreek	*Bulinus truncates*	1384	kaempferol (Omezzin*et al.*, 2014).	Abdallah*et al.*, (2011)
7	*Jatrophacurcas L.* (Co&m) (Co) (Co&Aq)	Euphorbiaceae	Physic nut	*Biomphalaria glabrata*	5, 50 500	Jatrophine (Patil*et al.*, 2013).	Rug and Ruppel (2000).
	(Co&m) (Co&Aq)			*Bulinus natalensis*	0.2, 750,		
	(Co&m) (Co&Aq)			*Bulinus truncates*	1, 750		
8	*Balaniteaegyptica*(s&Aq) Ec&Aq)(Mc& Aq)(wf&Aq)	Zygophyllaceae	Desert date	*Biomphalaria pfeifferi*	56.32 77.53 62.51 66.63	Diosgenin (saponin), (Al-Ghannam*et al.,* 2013).	Molla*et al.*, (2013)
9	*Moringaoleifera* (dl&E) (dl&Aq)	Moringaceae	Moringa or Drum stick tree	*Biomphalaria pfeifferi*	488.7 1057.0	Momordicine (alkaloid), (Hassan, 2013)	Asemota*et al.*, (2015).
10	*Sennaalata* (dl&E) (dl&Aq)	Fabaceae	Candle bush	*Biomphalaria pfeifferi*	474.8 753.0	Chrysophanol	Asemota*et al.*, (2015).
11	*Caesalpiniapulcherrima*(dl&E) (dl&Aq)	Fabaceae	Pride of Barbados	*Biomphalaria pfeifferi*	408.5 712.7	Azaleatin (flavanoid) (Rahman*et al.*, 2012).	Asemota*et al.*, (2015).
12	*Vernoniaamygdalina*(dl&E) (dl&Aq)	Asteraceae	Bitter leaf	*Biomphalaria pfeifferi*	338.8 614.8	Myricatin (Rahman*et al.*, 2012).	Asemota*et al.*, (2015).
13	*Alternentherasessilis*(dl&un) (dl&Ev) (fl&un) (fl&Ev)	Amaranthaceae	Sessile joy wood or dwarf copper leaf	*Bulinus globosus*	40.42 48.07 32.57 43.57	3β-O (b-D-glucopyranosyluronic acid) (Rahman*et al.*, 2012).	Azare*et al.*, (2007).

14	*Cymbopogoncitratus* (dl&E) (dl&Aq	Poaceae	Lemon grass	*Biomphalaria pfeifferi*	61.79 140.7	Azaleatin (Rahman*et al.*, 2012).	Otarigho and Morenikeji, (2012).
15	*Chromolaenaodorata* (dl&E) (dl&Aq	Asteraceae	Siam weed	*Biomphalaria pfeifferi*	88.04 217.57	Isorhamnetin (Rahman*et al.*, 2012).	Otarigho and Morenikeji, (2012).
16	*Terminaliacatappa* (dl&E)	Combretaceae	Tropical almond	*Biomphalaria pfeifferi*	864.1	Kaemferol(Rahman*et al.*, 2012).	Adetunji and Salawu, (2010).
	(dl&E)			*Bulinus globosus*	1095.7		
17	*Carica papaya* (dl&E)	caricaceae	Pawpaw	*Biomphalaria pfeifferi*	2716.3	Chymopapein and papain (Musa, 2015).	Adetunji and Salawu, (2010).
	(dl&E)			*Bulinus globosus*	619.1		
18	*Securidicalongependunculata* (dl&m) (Sb&m) (r&m) (dl&E) (Sb&E) (r&E)	Polygalaceae	Violate tree	*Bulinus globosus*	0.55 0.60 0.21 0.15 0.19 0.18	Securidacaxanthone A. (Lannang*et al., 2006*).	Olafintoye, (2010).
19	*Tephrosia bracteolate*(dl&m) (Sb&m) (r&m) (dl&E) (Sb&E) (r&E)	Fabaceae	Hoary pea	*Bulinus globosus*	0.18 0.30 0.44 0.19 0.45 0.35	Trans-tephrestachin (flavanon) (Khalid an Waterman, 1981 in Touqeel*et al.*, 2013)	Olafintoye, (2010).
20	*Talinumtriangulare*(rt)&E	Portulacaceae	Waterleaf	*Bulinus truncates*	300	Unknown	Okeke and Ubachukwu, (2011).
21	*Azadirachterindica* (dl&Aq) (dl&m) (dl&EAc)	Meliaceae	Neem tree	*Biomphalaria pfeifferi*	4397.20 1560.64 2000.21	Azadirachtin (Lakshmi *et al.,* 2015)	Syombua*et al.*, (2013).
22	*Entadaleptostachya* (r&Aq) (r&m) (r&EAc)	Fabaceae		*Biomphalaria pfeifferi*	3678.90 40.93 4397.20	Unknown	Syombua*et al.*, (2013).
23	*Zingiberofficinale* (rz&Aq) (rz&m)	Zingiberaceae	Ginger	*Bulinus globosus*	NMR, 214.72	[6]gingirol, (Pragassam*et al.*, 2011)	Labe, *et al.*, (2012).

KEYS

S= seed, Ec= endocarp, Mc=mesocarp ,wf= whole fruit, dl = dry leaf, fl = fresh leaf, Sb = stem back, r=root , rt = root tuber, rz=rhizome Ev = evapourated crude water extract, un= unevapourated crude water extract, Co= crude oil, Aq= aqueous extract, *m*= methanol extract, E = ethanol, EAc = ethyl acetate. Cw = cold water extract, A= adult snail, J= juvenile snail, O= ova, NMR= no mortality recoded

Table 1 present several plants specie that has been investigated for their molluscicidal properties. From the account of the plant molluscicides reviewed, with reference to Labe et al., (2012) keys of rating LC_{50}mollscicidal activity of plant as; (very strong molluscicidal activity, 0.1-10ppm, moderate to strong=50-100ppm, mild to moderate =100-200ppm, weak to mild = 200-400ppm) the following points can be deduced.

Securidicalongependuculata, and tephrosiabracteolate can be termed as very strong molluscides.
Euphorbia splendens, atriplexstylosa, jatrophacurcas, alternanthrasesselis, cymbopogoncitrus and balanitesaegyptica, as strong to moderate molluscicides. Guayacumofficinalis, talinumtriangulare and chromolaenaodorata, as moderate to mild molluscicides. Calotropisprocera, vernoniaamygdalina, nicotianatobacum, zingiberofficinale and trigonellafoenum, as weak to mild molluscicides.

Factors That Affect Efficacy of Molluscicides

1. **Organismal factors (snail factor)**

Size of snail play an important role in the absorption of plant extracts, adult snail has been shown to be more susceptible snail lethality than the juvenile snail (Rawi et al.2011), Newly-hatched (shell diameter < or = 1 mm) as well as young (3-8 mm) snails were slightly less susceptible than older (10-25 mm) mollusks. This could be attributed to large surface area to body ration in adult snails that exposes snail to larger amount of plant extract.

Detoxification of substance in snail body, upon encounter with toxic substance snails make a heroic last-ditch attempt to detoxify its body by producing more mucus, trying to excrete the noxious substance through its slime (Brook, 2013). This was earlier explained by Clark and Appleton (1996), that snails produce excessive mucus upon encounter with toxin. The mucus serve as a protective barrier preventing direct contact between the toxin and the epithelial of the skin or digestive tract thereby hindering the efficacy of the molluscicide (Henderson and Triebskorn, 2002).

2. **Chemical factors**

   a. **Presence of bioactive substance**

This is inadequately understood in terms of therapeutic action and it distribution within the plant. Obviously when undertaking an investigation of plant to obtain the active substance, it is impossible to isolate the entire constituent. Among the thousands of different substance, one or few are responsible for the therapeutic action (or the toxicity activity). Another factor is lack of information on the distribution of the molluscicidal activity present in different plant parts; such information might have a predictive value, but is not available due to failure of most investigators to systematically study all part of plant (WHO, 1983). Efforts have been made toward searching for bioactive substance responsible for molluscicidal compound in plants, as indicated in (table 1). Further investigation on bioactive compound in plant that shows molluscicidal potency may help to explain variation obtained in the results obtained by different investigators.

b. Presence of mixed function oxidases

Detoxification is done by a system of mixed function oxidases (MFO) that oxidase, reduce and hydrolyze toxic chemicals into soluble molecules that can be eliminated. MFO is a general detoxifying agent, and it is induced into activity by a wide range of toxic chemicals.MFO is an enzyme complex found in animals that oxidize toxic compounds to render them more susceptible to metabolism and excretion. The enzyme is localized in hepatopancrease in invertebrates (El Gohary et al., 2011). Studies carried out by El Gohary et al.,(2011) indicated that metaldehydecompounds (gastrotox and mlotov) caused increase in the level of MFO in both tested land snailseobania. vermiculataand monocha. cantiana, while the level of this enzyme was decreased in the two treated land snails, when applied with carbamate compound (mesurol). Henderson and Triebskon (2002) explained that MFO serve as the leading step in the detoxification process to limit damage by toxin or excrete them out by biotransformation of chemical through oxidation.

3. Laboratory conditions and experimental technique

Results of biological tests of plant molluscicide often vary and depend on experimental conditions such as temperature, concentration of organic material in water, materials, models taken for biological experimentation (age, life cycle), and method of extraction of plant extracts among others. For instance Oliveira-Filho et al., (1999) showed that molluscicidal effect of E. milii latex was modified by environmental factors such as temperature (i.e., LC50 and LC90 values were halved for every 10^0C rise in temperature) Higher activity of plants extract at higher temperature was attributed to increase release and solubility of active constituents of plant at hot medium, this causes easy penetration of active ingredient to the snail membrane (Hamed et al., 2015).

Presence of organic particle such as mud decreases the activity of plant extract. This is due to adsorption of active ingredient of the extractant on mud (Hamed et al., 2015). Dissolve oxygen is also one of the factors which alter the toxicity of plant extract against snails. Singh et al., (2010b) recorded less mortality of lymneaacuminata exposed to oleoresin during the winter compared to the summer period. This was attributed to the fact that water in winter holds much oxygen. At high temperature dissolve oxygen concentration decrease which is reflected by high mortality of snail.

Mode of Action of Plant Molluscicide on snails

Investigations on mode of action plant molluscicide are done to discover which molluscan systems are affected by molluscicide. The importance of understanding the mode of action of plant molluscicides has been stressed by various investigators (WHO, 1983), in hope that less toxic, cheaper, readily available molluscicide that could be used in control of snail intermediate host of various parasitic disease could be obtained. Although effective, molluscicidal showing the activity of some compounds has not proved entirely satisfactory (Singh *et al.*, 2010). In an attempt to understand the mode of action plant molluscicide that leads to snail death, investigation were made by few researchers and their findings are discussed below. Based on their mode of action, plant molluscicide can be categorized into following categories; enzyme inhibitors, neurotransmitters inhibitors (or neuro toxin), stomach poisons, respiratory poisons, contact poisons and growth inhibitors.

Interference with snail enzymes (poison of enzyme)
Acetylcholinestrase (AchE) is a key enzyme in the nervous system of animals, the enzyme occurred in the outer basal lamina of nerve synapse neuromuscular junction and certain other tissues (Akinpelu et al., 2012). The enzyme is responsible for the termination of cholinergic impulse by hydrolysis of acetylcholine (Ach) released during synaptic transmission (Singh and Singh, 2003). The mechanism of action of AchE as described by Sharaf El-Din et al., (2012) is that AchE is released at the myoneutral junction in organisms if an action potential is developed at the nerve ending and diffuses through the gap between the nerve and the muscle (the gap is about 100 A° wide). Inhibition of AchE thus permits accumulation of Ach at the synapses which concentration raises several folds in comparison to the normal levels leading first to paralysis and then eventually death of the snail (Singh and Singh, 2003). It was postulated that the inhibitor (pesticide) serves as pseudosubstrate and become attached to the active site of the enzyme. The hydrolysis of the inhibited enzyme is slow and therefore the amount of AchE becomes lesser which lead to accumulation of acetylcholine at the nerve endings (Rawi et al., 2011). Achieving acute toxicity is the goal for an effective molluscicide, the early phase of metabolic activation is usually followed by a decrease and finally a cessation of enzymatic activity coincident with cell and animal death (Henderson and Triebskorn, 2002).

Interference with neuron (neurotoxins)
Sharaf El-Din et al., (2012) extensively studied the mode of action and neuropathological effect of selecron, bayluscide and ethanolic extract of anagalisarvensison the neurons of the cerebral gangliain the freshwater snail B. alexandrina.Electron microscopical examination of treated animals revealed severe ultra-structural alterations in the cerebral ganglia. These alterations included hyperchromatic, pyknotic or highly shrunken nuclei, extreme indentation of plasma membrane, atrophy of the perikarya of some neurons, margination of nucleoli, fragmentation or dilation of rough endoplasmic reticulum, damage of mitochondria and vacuolation and destruction of cytoplasm. The consequences of this distortion result in caseation of cellular activities such as protein synthesis. Proteins are critical chemical compounds that control everything that cell do, in addition they make up the material from which cell and cell parts themselves are made. Anagalisarvensis are termed as neurotoxin because of its affinity to the ganglia which serve as the nervous center for the mollusk. The nature of the molluscan nervous system is such that any damage caused to it could result in a wide range of effects, e.g. changes in heart rate, oxygen consumption and water uptake (Clark and Appleton, 1996). Bioactive compound that has been reported to cause muscular weakness, paralysis and death in snails was iso-pelletierine (alkaloid) isolated from the bark stem of punicagranatum (Tripathi and Singh, 2000).

Interference with digestive system
Saponins extracted from many source has been reported to exhibit molluscicidal properties (table 2) the target sites of saponins include muscle, haemolymph, intestine and hepatopancrease (stomach poison) of fresh water snail. It inhibits the activity of AchE in the internal tissue of the snail (Akinpelu et al., 2012). Plant extract of Euphorbia splendens, ziziphusspina-christi and ambrosia maritime were reported to induce histopathological effects of the on digestive gland of Biomphalaria alexandrina and Bulinus truncates.The damage, included vaculations, disappearance of secretory cells from the digestive tubules as well as connective tissue between shrinked acini and accumulation of the toxic agents in the cytoplasm of digestive and excretory cells (Bakry, 2009b; Ahmed et al., 2012). Thereby altering the permeability of the cell or interfere with regulatory or metabolic activities within them, (Bakry, 2009).

Interference with growth (growth toxins)

Some molluscicide are growth inhibitors as they Interfere with growth rate of the snails, this is evidenced from the investigation of Bakry, (2009a), that revealed the effect of methanolic extract of three plants Euphobiasplendens, Atriplexstylosa and Guayacumofficinalis on Biomphalaria alexandrina snail which showed considerable effect on the growth of snails by comparable decrease in shell size in the treated snails measuring, 5.22mm, 5.8mm and 6.2mm at the three week expose to LC_{25} which is lower than the control group (7.4mm). Reduction in growth rate could also be attributed to interference of plant extract with physiological activities of the snails i.e. activities of enzymes in tissues and hemolymph of treated snails (Bakry, 2009a). Some plant extracts have influence on feeding behavior of the snail. A study conducted on Jatrophagossypifolia against B.glabrata shows low food consumption of snail in contact with the extract from leaves and fruits this hinder the locomotive ability of snail to actively seek for food which is the fundamental for development and proliferation, and when its affected this can lead to losses with regard to establishment of snail population (Pereira et al., 2014).

Interference with osmoregulation

Osmoregulation is a physiological process that organism uses to maintain water balance i.e. to compensate for water loss, avoid excess water gain and maintain proper osmotic concentration of the body fluids. Given that the haemolymph of freshwater pulmonates is hyper-osmotic to the external medium and that most molluscan tissues are highly permeable, maintenance of water balance must be continuous, thus any compound interfering with the mechanisms of maintaining water balance will therefore lead to a rapid increase or decrease in body weight (Clark and Appleton, 1996).The swelling of body tissues following the application of molluscicides has been suggested as a failure of water balance control (Clark and Appleton, 1996). Labe et al., (2012) reported similar incidence on Bulinus globusus exposed to methanolic extract of zingiberofficinale, which result in development of hemorrhagic blisters over the foot sole of the snail with visible swelling of the cephalopodal mass. This resulted from damaged epithelial surfaces and consequently increases the permeability of epithelial membranes and accumulation of water in tissues, and haemorrhage thus, preventing its normal osmoregulatory function. Such kind of damage may also lead to loss of haemolymph, and hence body water, however, this will cause loss rather than uptake of water. Other factors that could lead to water imbalance in snail exposed to molluscicide; are impairment of kidney function, damage to the ganglia or neurosecretion and excessive production of mucus when the snail encounters the toxin and distress syndrome (Clark and Appleton, 1996; Labe *et al.*, 2012).

Table 2. Mode of Action of Some Plant Molluscicide

Mode of action of the plant extract	Bioactive compound	Plants	Investigators
Inhibition of enzymatic activities	Alkaloids (iso-pelletierine), saponin	*Euphorbia splendens, Atriplexstylosa, Guayacumofficinalis, zingiberofficinale, Cymbopogoncitratus,Erythrophleumsuaveolens*	Bakry, (2009), Akinpelu *et al.*, (2012) Labe, et al., (2012).
Interference with growth rate	Not known	*Chromolaenaodrota, Euphorbia splendens, Atriplexstylosa, Guayacumofficinali*	Bakry, (2009a), Otarigho and Morenikeji, (2012).
Reduction in cercarial shedding period	Mechanical injury in the presence of lethal plant extracts	*Euphorbia splendens, Atriplexstylosa*	Bakry, (2009a).
Distress syndrome	Water inbalance in the presence of plant extracts	*BalaniteaegypticaSennaalata, Caesalpiniapulcherrima, Vernoniaamygdalina, Alternentherasessilis, Cymbopogoncitratus, Chromolaenaodorata, Terminaliacatappa, Carica papaya, Securidicalongependunculata , Tephrosia bracteolate, Talinumtriangulare, Azadirachterindica, Entadaleptostachya*	Adetunji and Salawu, (2010), Olafintoye, (2010), Okeke and Ubachukwu, (2011), Otarigho and Morenikeji, (2012), Syombua et al., (2013).

Conclusion

This review, are few numbers of plants extract and constituent that have molluscicidal effect on the snail intermediate host of schistosome. Most of the plant listed had very strong LC_{50} on experimental snails this may be attributed to the presence of bioactive substance such as alkaloids and saponins that interferes with metabolic activities in snail vectors in number of ways such as interference with enzymatic activities, interference with neuron transmission, interference with digestive system, interference with osmoregulation and growth leading to various pathological effect on cells and organs of the snails, decrease in size, paralysis and subsequent death of the animals. However, there are some factors that could hinder the efficacy of plant molluscicides this could be related to snail defense mechanism such as mucus production, presence of MFO, size or age of the snail. It could also be due to absence or in adequate bioactive substance from plant materials than could cause the death of the snail. Other factors are laboratory and experimental techniques in handling and testing the molluscicidal activities such as problem with extraction and isolation of bioactive substance from plant as well as testing them against the snail vectors.

Recommendations

1. There is inadequate information on bioactive substance presence in plants that are responsible for molluscicidal action, this necessitate an improve study on the isolation of bioactive substance from most folkoric medicinal plant that has been proven to be effective against the snail intermediate host of *schistosoma* and *Fasciola* this should also involve various parts of the plant.
2. There is need to for improve study on the mode of action of plants that are responsible for molluscicidal action. Understanding the mode of action of plant molluscicidal is vital in limiting the toxicity against non-target organisms.

3. It is possible that using other extraction technique such as soxhletextractor could yield a better lethal concentration of the plant at much lower dose, alongside with improve experimental conditions.

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