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Galleria mellonella (greater wax moth) as an eco-friendly in vivo approach for the assessment of the acute toxicity of medicinal plants: Application to some plants from Cameroon

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Abstract

Background: The evaluation of medicinal plants' toxicity is a prerequisite prior to their usage. The vertebrate models used for this purpose are often the object of ethical consideration. Though invertebrate models including *Galleria mellonella (GM)* have demonstrated the ability to be used to assess the toxicity of various products. To the authors' knowledge, *GM* has never been exploited to determine the toxicity of medicinal plants.

Aim: The aim of this study was to demonstrate the potential of *GM* larvae as a simple, inexpensive, and rapid model for the evaluation of the toxicity of herbal medicines.

Method: In this study, the toxicity of aqueous and ethanolic (80%, v/v) extracts of seven well known plants from Cameroon namely leaves of *Cymbopogon citratus* (DC.) Stapf, *Moringa oleifera* Lam and *Vernonia amygdalina* Delile; barks of *Cinchona officinalis* and *Enantia chlorantha* Oliv; barks and seeds of *Garcinia lucida* Vesque and leaves and seeds of *Azadirachta indica* (Neem) was evaluated using the larval form of *GM*. The median lethal doses (LD_{50}), 90% (LD_{90}), and 100% (LD_{100}) were determined using the spline cubic survival curves and equations from the data obtained on the survival rate of *GM* 24 hours after the injection with the extracts.

Results: We found that distilled water extracted a more important mass of phytochemical compounds (7.4%–21.2%) compared to ethanolic solution (5.8%–12.4%). LD varied depending on the plant materials and ethanolic extracts (hydroalcoholic extract, (HAE)) were more toxic to *GM* than aqueous ones. The LD_{50} (mg/ml) of the tested extracts varied from 4.87 [3.90 g/kg body weight (bw)] to >200 (> 166.67 g/kg bw), the LD_{90} (mg/ml) from 25.00 (18.52 g/kg bw) to >200 (> 181.82 g/kg bw) and LD_{100} (mg/ml) from 45.00 (40.91 g/kg bw) to > 200 (>181.82 g/kg bw). The HAE of *A. indica* seed and *C. officinalis* bark exhibited the highest toxicity with LD_{50} (g/kg bw) of 3.90 and 4.81, respectively.

Conclusion: The results obtained in this study suggest that *GM* can be used as a sensitive, reliable, and robust eco-friendly model to gauge the toxicity of medicinal plants. Thus, avoid the sacrifice of vertebrate models often used for this purpose to limit ethical concerns.

Keywords: Medicinal plants, Greater wax moth, *Galleria mellonella*, Insect model, Toxicity.

Introduction

Medicinal plants have been used for millennia to treat various maladies including respiratory tract infections, urinary tract infections, sepsis, diabetes, erectile dysfunction, diarrhea, fevers and as a stabilizer of physiological functions among many others (Kuete *et al.*, 2010; Sylvie *et al.*, 2014). In this study, the herbal medicines used were leaves of *Cymbopogon citratus* (DC.) Stapf, *Moringa oleifera* Lam, and *Vernonia amygdalina* Delile; barks of *Cinchona officinalis* and *Enantia chlorantha* Oliv; barks and seeds of *Garcinia lucida* Vesque and Leaves and seeds of *Azadirachta indica* (Neem). These medicinal plants are among the most popular in Cameroon (Kuete *et al.*, 2010; Sylvie *et al.*, 2014; Sonfack *et al.*, 2021)

Cinchona officinalis is a shrub of the Rubiaceae family whose bark is recognized for its very bitter taste and its composition in alkaloids, such as quinine, dihydroquinine, cinchonidine, epiquinine, quinidine, dihydroquinidine, cinchonine, and epiquinidine (Júnior *et al.*, 2012; Bharadwaj *et al.*, 2018). In addition to its antimalarial properties, *C. officinalis* has also been used on a smaller scale as a treatment for goiter, Meniere's disease (an inner ear disorder), varicose veins, and in obstetrics for its action on uterine muscles (Júnior *et al.*, 2012).

Garcinia lucida Vesque (*G. Lucida)* is also a well-known herbal medicine whose properties and composition have been well-studied. The seed, fruit, and bark are the most commonly used parts in traditional medicine and food (Sylvie *et al.*, 2014). Used in fermentation of palm tree or raffia wine (Guedje *et al.*, 2017), the bark and seeds have been found useful in the treatment of gastric and gynecological infections, diarrheas, cure for snake bites as well as an antidote against poison (Sylvie *et al.*, 2014). In addition, a recent study conducted by Sonfack *et al.* (2021) revealed that the aqueous extract from the

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stem bark of *G. lucida* exhibits cardioprotective and nephroprotective effects in adenine-induced chronic kidney disease in rats. This plant is also believed to possess some aphrodisiac properties and also used in ghost-hunting practices (Guedje and Fankap, 2001). Investigations on the chemical composition of *G. lucida* have shown that it contains the terpenoids and xanthones products such as 1,2-dihydroxy-xanthone, 1-hydroxy-2-methoxyxanthone, putranjivic acid, methyl putranjivate, and friedelin (Kuete *et al.*, 2010; Sylvie *et al.*, 2014).

Enantia chlorantha Oliv (*E. chlorantha*) is a plant belonging to the Annonaceae family with multiple uses in traditional medicines. This versatile plant is also referred to as Epoue (Baka), Peye (Badjoue), and Nfol (Bulu). It is widely spread along Sub-Saharan Africa (Etame *et al.*, 2019). In Cameroon, *E. chlorantha* is used in the management of various infections including malaria, typhoid fever, jaundice, dysentery, wounds, high blood pressure, urinary infection, leprosy spots, and convulsions. Its yellow, dried, or fresh bark has been reported to have antiviral (Ohemu *et al.*, 2018), antifungal (Abike *et al.*, 2020), and antibacterial properties (Etame *et al.*, 2019; Abike *et al.*, 2020). Other studies have reported its use as an antioxidant and antipyretic (Olivier *et al.*, 2015). Investigations into its composition revealed that *E. chlorantha* contains numerous biologically active compounds, the most important of which are alkaloids such as protoberberines (berberine, canadine, palmatine, jatrorrhizine, columbamine, and pseudocolumbamine), phenanthrene alkaloids (atherosperminine and argentinine), and aporphines (7-hydroxydehydronuciferine and 7-hydroxydehydronornuciferine) (Olivier *et al.*, 2015).

Azadirachta indica (*A. indica)* known as Neem is a monoecious tree of the Meliaceae family whose oil produced from its seeds is widely used for its medicinal properties in the northern part of Cameroon. It is known that compounds in Neem extracts have anti-inflammatory, anti-hyperglycaemic, anti-carcinogenic, antimicrobial, immune-modulator, anti-mutagenic, antioxidant, anti-ulcer, and anti-viral effects (Arévalo-Híjar *et al.*, 2018; Baildya *et al.*, 2021). Recent studies by Baildya *et al*. (2021) found 19 compounds from this plant which may be used to fight against COVID-19. In addition, Azadirachtin, one of its major constituents, gives it insecticidal properties which acts by intercepting metamorphosis from the larval to adult stage, and paralyzes the digestive tract of the larva (Tofel *et al.*, 2017; Meisyara *et al.*, 2019). The different parts of *A. indica* have been widely used in recent years in the green synthesis of nanoparticles such as silver nanoparticles (AgNPs), Zinc oxide nanoparticles (ZnONPs), gold (AuNPs) and copper nanoparticles (CuNPs) (Nagar and Devra, 2018; Abbas *et al.*, 2020; Vijayakumar *et al.*, 2020; Chinnasamy *et al.*, 2021).

Moringa oleifera Lam (*M. oleifera*) is both an edible and a medicinal plant. Every part of the plant, from the leaves to the roots, has been reported to possess potential health benefits (Aderinola *et al.*, 2020). This plant belonging to the Moringaceae family contains a profile of important minerals, and is a good source of protein, vitamins, β-carotene, amino acids, and various phenolic compounds (Anwar *et al.*, 2007). *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, β-sitosterol, caffeoylquinic acid, and kaempferol (Anwar *et al.*, 2007; Aderinola *et al.*, 2020). Besides its nutritional properties, *M. oleifera* is traditionally used to treat skin infections, asthma, diabetes, diarrhea, arthritis, inflammation, cough, fever, and headache. It has also been reported to have antioxidant, anti-inflammatory, antitumor, antimicrobial, hepatoprotective, and anti-arthritic properties (Ray *et al.*, 2015; Arulselvan *et al.*, 2016; Saleem *et al.*, 2020).

Like *M. oleifera, V. amygdalina* Delile (*V. amygdalina*) is both an edible and a medicinal plant. *Vernonia amygdalina* belongs to the family Asteraceae and is called bitter leaf in English because of its bitter taste. In Cameroon, *V. amygdalina* is known under the name of Ndolè and is included in the composition of the national popular dish which bears the same name. The presence of polyphenols, vitamins, and mineral salts makes the plant useful in human diets (Tonukari *et al.*, 2015; Dumas *et al.*, 2021). Moreover, this plant is rich in phytochemicals such as saponins, sesquiterpenes, flavonoids, and steroid glycosides (vernosides), lactones (Dumas *et al.*, 2021) and several studies reported that it has anticancer and antitumor activity (Hasibuan *et al.*, 2020; Joseph *et al.*, 2020); antihepatotoxic activity (Yedjou *et al.*, 2018); hypoglycemic activity (Dumas *et al.*, 2021); antibacterial activity (Egbuonu and Amadi, 2021); anti-inflammatory (Wang *et al.*, 2020) as well as antioxidant property (Alara and Abdurahman, 2021).

Cymbopogon citratus (DC.) Stapf (*C. citratus*) or lemongrass is a herbaceous plant of the Poaceae family growing in humid tropical areas which is mainly cultivated for its stems and leaves whose infusion gives a tea with strong lemony odor due to its high content of the aldehyde citral. (Do *et al.*, 2021). Muala *et al*. (2021) reported that lemongrass is rich in minerals, vitamins, macronutrients (including carbohydrate, protein, and small amounts of fat) and its leaves are a good source of various bioactive compounds such as alkaloids, terpenoids, flavonoids, phenols, saponins, and tannins that confer *C. citratus* leaves pharmacological properties such as anti-cancer, antihypertensive, anti-mutagenicity, anti-diabetic, antioxidant, anxiolytic, anti-nociceptive, and anti-fungi. *Cymbopogon citratus* is also commonly used in folk medicine for treatment of nervous and gastrointestinal disturbances, and as an antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic, and a sedative (Hanaa *et al.*, 2012).

In general, medicinal plants constitute a source of new biologically active molecules that are economically accessible to deal with the emergence of phenomena of

resistance of germs to chemical molecules (Koné *et al.*, 2020). The common point of all these medicinal plants (and all the molecules that result from them) that can potentially be used in the treatment of various pathologies is the evaluation of their toxicity. Indeed, toxicity testing in rodents is an important prerequisite to the use of compounds in man (Ignasiak and Maxwell, 2017). However, trials in rats and mice are expensive, not eco-friendly and there are ethical considerations (Ignasiak and Maxwell, 2017). In this context, invertebrate models appear as a very promising alternative and have seen a gain in popularity in recent years (Megaw *et al.*, 2015; Ignasiak and Maxwell, 2017; Allegra *et al.*, 2018; Wijesinghe *et al.*, 2020; Moya-Andérico *et al.*, 2021). The most used invertebrate models for toxicity trials among others are *Artemia salina*, *Daphnia magna*, *Drosophila melanogaster*, soil invertebrate *Folsomia candida*, the nematode *Caenorhabditis elegans*, and the greater wax moth *Galleria mellonella* (*GM*) (Megaw *et al.*, 2015; Ogungbemi and van Gestel, 2018; Land *et al.*, 2020; Zhang *et al.*, 2021). Among all these *in vivo* insect models, *GM* has a number of attractive benefits due to its practicability, in particular its low cost, ease of acquisition and handling (no specialist training or equipment is required), ability to survive at 37°C (unlike many other non-mammalian hosts), they are large enough (2 cm in length and weigh approximately 250 mg) for accurate dosing (unlike commonly used invertebrate models such as *C. elegans* and *D. melanogaster*) which make it possible to handle individual larvae and administer test compounds directly into each individual (which is not always possible with other smaller invertebrate models) and enhances the reproducibility of assays conducted using this model (Megaw *et al.*, 2015; Ignasiak and Maxwell, 2017; Piatek *et al.*, 2020).

The aim of this study was to demonstrate the potential of *GM* larvae as a simple, inexpensive, and rapid model for the evaluation of the toxicity of herbal medicines such as *C. officinalis, G. lucida* Vesque, *E. chlorantha* Oliv, *A. indica* (Neem), *M. oleifera* Lam, *V. amygdalina* Delile, and *C. citratus*. To the authors' knowledge, this study presents the first use of *GM* for an *in vivo* lethal doses (LD_{50}) study of medicinal plants.

Material and Methods

Plant material

The plant material used in this study were leaves of *C. citratus* (DC.) Stapf, *M. oleifera* Lam and *V. amygdalina* Delile; barks of *C. officinalis* and *E. chlorantha* Oliv; barks and seeds of *G. lucida* Vesque and leaves as well as seeds of *A. indica* (Neem). These plants were chosen because they are renowned for their use in traditional medicine in Cameroon and because of data existing in the literature on their effectiveness and their composition. All the plants were collected in September 2020 in different regions of Cameroon. *Vernonia amygdalina and C. citratus* was collected in Nlobison II, Central region of Cameroon (VJ5J + C3 Yaounde, Cameroon); *C. officinalis*, *E. chlorantha*, and *G. lucida* were bought in the Nkoabang Market (VH7M+FJ Yaoundé, Cameroun); *M. oleifera* was bought in the Dang Market of Adamaoua region (CHH5 + 67 Ngaoundéré, Cameroun) and *A. indica* was bought in the North region (7CX2 + X4 Garoua, Cameroun). The collected plants were dried at room temperature in the shade for 7 days then packaged in hermetically sealed plastics and additional packaging was done to facilitate shipment to Russia in December 2020 where they were received by the Laboratory of Microbiology, Faculty of Medicine of the RUDN University. Plants were grinded and the powders with particle sizes lower than 1 mm were stored in a sterile airtight container until further use.

Galleria mellonella

GM larvae were obtained commercially from https://ecobaits.ru/ (ECO BAITS, Moscow, Russia) and stored at 15°C prior to use. Dead larvae and those with dark spots or showing signs of melanization were discarded.

Extraction of active compounds

Ethanolic solution (80%, v/v) and distilled were used because they have been reported to be efficient solvents for the extraction of bioactive compounds in the medicinal plants used in this study. As we described in our previous investigation (Mbarga *et al.*, 2021), 50 g of plant material was weighed and added to 450 ml of the solvent in separate conical flasks. The flasks were covered tightly and were shaken at 200 rpm for 24 hours and 25°C in a shaker incubator (Heidolph Inkubator 1000 coupled with Heidolph Unimax 1010, Germany). The mixtures were then filtered by vacuum filtration, using Whatman filter paper № 1 then concentrated at 40°C in rotary evaporator (IKA RV8) equipped with a water bath IKA HB10 (IKA Werke, Staufen, Germany) and a vacuum pumping unit IKA MVP10 (IKA Werke, Staufen, Germany). In order to avoid losses, the extracts were collected when the volumes were small enough and placed in Petri dishes previously weighed and then incubated open at 40°C until complete evaporation. The final dried crude extracts were weighed. Extract volume and mass yield were determined using the following formulas:

$$\text{Volume yield (\%)} = \frac{\text{Volume of the extract after filtration (ml)}}{\text{Initial solvent volume (ml)}} \times 100$$

$$\text{Mass yield (\%)} = \frac{\text{Mass of extracted plant residues (g)}}{\text{Mass of plant raw sample(g)}} \times 100$$

Stock solutions

One gram of each crude extract was dissolved in 5 ml of sterile phosphate-buffered saline (PBS) to obtain

the concentration of 200 mg/ml. These solutions were sterilized by microfiltration (0.22 µm) and dilutions were performed in sterile PBS to obtain concentrations of 150, 100, 50, 25, 12.5, and 5 mg/ml. The sterile PBS plant extract free was used as control. All the eight tests solution were stored at 4°C.

Toxicity assay

The larvae were weighed and only those from 0.2 to 0.5 g were retained. For each concentration of extract, 3 groups of 20 randomly selected *GM* larvae were used (Fig. 1A). 20 µl of each dilution were injected using a 0.3 ml Terumo® Myjector® U-100 insulin syringe (VWR, Russia) through the base of the last left proleg as described by Megaw *et al*. (2015), and shown in Figure 1B. Control groups of 10 larvae injected with 20 µl sterile PBS were also included. After 24 hours of incubation in the dark at 37°C, larvae were examined for mortality, and were considered dead if they were unmoving, failed to reorient themselves when placed on their backs, and failed to respond to stimuli as indicated by Megaw *et al*. (2015). Percentage survival was plotted as a function of concentration for each plant extract sample using Spline cubic model in the statistical software XLSTAT 2020 (Addinsof Inc., New York) and median LD_{50}, LD90 and LD_{100} values expressed in mg/ml were calculated using each specific spline cubic equation or spline cubic curves obtained. Spline curves were used when the fit accuracy of the spline equation was less than 90%). The mean weight of each group of 20 larvae was used to extrapolate the LD_{50}, LD_{90} and LD_{100} values for each plant extract sample in g/kg using the formulas:

$$\text{LD50(g/kg body weight)} = \frac{\text{Volume administered (ml)} \times \text{LD50(mg/ml)} \times 10^{-3}}{\text{Body weight (kg)}} \times 100$$

$$\text{LD90(g/kg body weight)} = \frac{\text{Volume administered (ml)} \times \text{LD90(mg/ml)} \times 10^{-3}}{\text{Body weight (kg)}} \times 100$$

$$\text{LD100(g/kg body weight)} = \frac{\text{Volume administered (ml)} \times \text{LD100(mg/ml)} \times 10^{-3}}{\text{Body weight (kg)}} \times 100$$

Results and Discussion

The extract yields obtained for the nine samples from our seven medicinal plants using ethanolic solution and distilled water are recorded in Table 1. As observed in Table 1, the highest volume yields were observed with ethanolic extracts while the highest mass yields were obtained with aqueous leaves extract of *M. oleifera* (21.2%), *C. citratus* (17.3%), *A. indica* (15.1%), and *V. amygdalina* (14.0%). The highest mass yield with ethanolic solution was obtained with *E. chlorantha* bark (12.4%). Extraction with ethanolic solution was less effective in *A. indica* seed and *M. oleifera* leaves with mass yields of 5.8% and 7.3%, respectively. In most of the studies where extraction of active compounds was performed, it was reported that water had a better extraction rate than other solvents such as ethanol or methanol (Arsene *et al.*, 2021; Mbarga *et al.*, 2021;

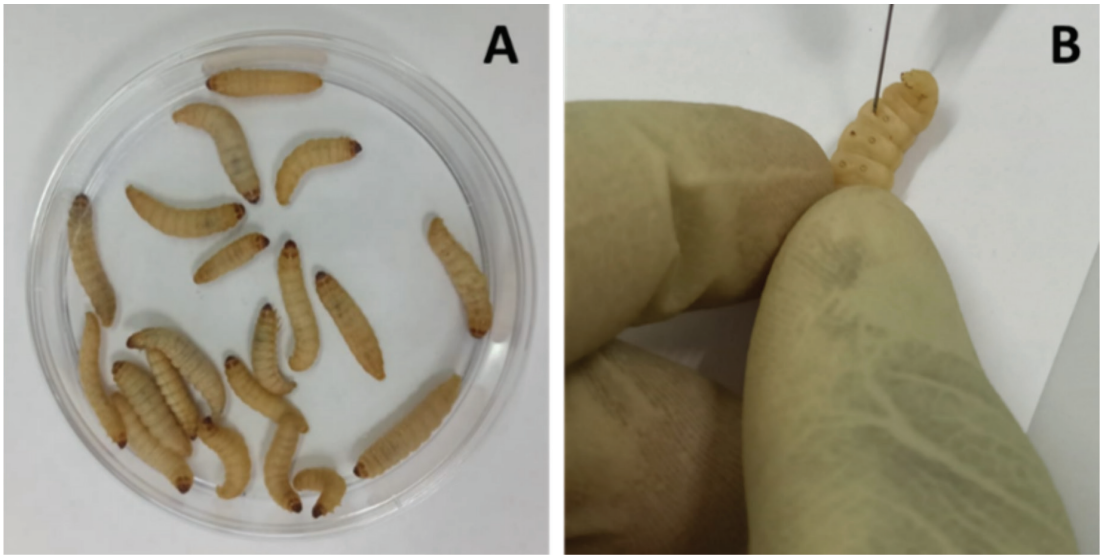

Fig. 1. (A): Group of 20 larvae. (B): Injection of *GM* larvae with plant extracts through the last left proleg.

Table 1. Extract yields (%) obtained from the seven plants with distilled water and ethanolic solution (EtOH 80%).

Medicinal plants	Parts used	Local name	Volume yield (%, v/v)		Mass yied (%, w/w)	
			EtOH extract	Aqueous extract	EtOH extract	Aqueous extract
Cinchona officinalis	Bark	Ikouk	95.0		8.9	9.7
Vernonia amygdalina	Leaves	Ndole	78.0	75.0	8.3	9.5
Garcinia lucida	Seed	Essok	93.0	90.0	9.8	14.0
	Bark		93.0	86.0	8.4	7.9
Enantia chlorantha	Bark	Nfol	92.0	85.0	12.4	9.3
Azadirachta indica	Leaves	Neem	87.0	83.0	5.8	15.1
	Seed		91.0	88.0	9.3	7.4
Moringa oleifera	Leaves	Moringa	82.0	77.0	7.3	21.2
Cymbopogon citratus	Leaves	Citronelle	87.0	86.0	8.3	17.3

Mouafo *et al.*, 2021). Mouafo *et al*. (2021) reported that this difference is mainly attributed to the polarity of the solvents and therefore to the large proportions of water-soluble compounds present in the plant. However, high yields of phytoconstituents obtained does not necessarily imply a high biological activity (Mouafo *et al.*, 2021). We observed this in our study through the (LD_{50}, LD_{90}, and LD_{100}) determined for each of the extracts of the medicinal plants tested. Indeed, as shown in Table 3, LD_{50}, LD_{90}, and LD_{100} values vary considerably from one plant to another but above all, we noticed that for all medicinal plants, the LD of ethanolic extracts were lower than those of aqueous extracts. This implies that ethanolic extracts contain more active compounds and are therefore more toxic than aqueous extracts because, as reported by Situmorang *et al*. (2020), low LD_{50} values are associated with high toxicity (high amount of active compounds) and conversely, high LD_{50} values are associated with low toxicity (low amount of active compounds).

It is important to remember that the LD values were obtained using the greater wax moth (*GM*) as a toxicity assessment model. After 24 hours of incubation, survival data for each concentration of each plant material was used to plot spline cubic survival curves (examples of which are shown in Fig. 2) and to obtain the spline cubic equations (examples of which are presented in Table 2). As expected, no deaths were observed in larvae injected with 20 µl of PBS. However, as shown in Table 3, the LD_{50} (mg/ml) of the extracts tested varied from 4.87 [90 g/kg body weight (bw)] to >200 (> 166.67 g/kg bw), the LD_{90} (mg/ml) from 25.00 (18.52 g/kg bw) to >200 (> 181.82 g/kg bw) and LD_{100} (mg/ml) from 45.00 (40.91 g/kg bw) to > 200 (>181.82 g/kg bw). This difference between the LD values confirmed the good sensitivity of *GM*, which was reported as changing depending on the substances tested (Megaw *et al.*, 2015; Ignasiak and Maxwell, 2017). The extracts with the highest toxicity were *C. officinalis* ethanolic bark extract (LD_{50} = 3.90 g/kg bw) followed by *A. indica* ethanolic seed extract (LD_{50} = 4.81 g/kg bw) and *C. officinalis* aquous bark extract (LD_{50} = 8.66 g/kg bw). However, other plant material such as water extract of leaves of *C. citratus*, *M. oleifera*, and *V. amygdalina* exerted the lowest toxicity on the greater wax moth with LD_{50} (mg/ml) of 149.08; 165.40 and > 200 respectively.

The high toxicity of both ethanolic and aqueous extract of *C. officinalis* bark can be attributed to its composition having abundant quinine and its derivatives (dihydroquinine, cinchonidine, epiquinin, quinidine, dihydroquinidine, cinchonine, and epiquinidine) (Júnior *et al.*, 2012; Bharadwaj *et al.*, 2018) which are slightly soluble in water and soluble in ethanol (Gaponenko *et al.*, 2020). Indeed, Quinine, $C_{20}H_{24}N_2O_2$, (8S, 9R) -6′-methoxycinchonana-9ol;(αR)-α-(6-methoxy-4-quinoyl)-α-[(2S, 4S, 5R)-(5-vinylquinuclidin-20yl)] methanol, is the most important alkaloid occurring in Cinchona (Maurice, 2014). We did not find any study in the literature evaluating the toxicity of extracts of *C. officinalis* but quinine was reported to have an LD_{50} of 1.392, 0.660 and 0.641 g/kg bw when administered orally to rats, mice, and rabbits, respectively (ECHA, 2021). These values differed to ours probably because the results reported by ECHA (2021) were those of pure quinine while our study focused on extracts with heterogeneous composition. Furthermore, and interestingly, we observed that some larvae exposed to concentrations greater than the LD_{100} of ethanolic extracts of *C. officinalis* bark exhibited localized melanization, whereas larvae injected by lower concentrations had slight and uniform melanization (Fig. 3). These results are similar to those obtained by Megaw *et al*. (2015) who used the *GM* model to test the toxicity of 1-alkyl-3-methylimidazolium chloride ionic liquids. Megaw *et al*. (2015) reported that melanization is a common component of the insect immune

Table 2. Cubic spline model used to determine the LD_{50}, LD_{90} and LD_{100} of *E. chlorantha bark* ethanolic extract (HAE), *C. citratus* leaves HAE, *A. indica seed* HAE and *G. lucida* bark HAE.

Plant material	Spline intervals	*t*^3	*t*^2	*t*	Constant	R^2
Enantia chlorantha bark HAE	(0; 5)	−0.005	0.000	0.119	100.000	0.92
	(5; 12.5)	0.002	−0.072	−0.239	100.000	
	(12.5; 25)	0.001	−0.028	−0.986	95.000	
	(25; 50)	0.000	0.005	−1.278	80.000	
	(50; 100)	0.000	0.000	−1.163	50.000	
	(100; 200)	0.000	0.010	−0.668	0.000	
Cymbopogon citratus leaves HAE	(0; 5)	0.003	0.000	−0.067	100.000	0.88
	(5; 12.5)	−0.008	0.040	0.133	100.000	
	(12.5; 25)	0.004	−0.133	−0.567	100.000	
	(25; 50)	0.000	0.019	−2.000	80.000	
	(50; 100)	0.000	0.011	−1.264	40.000	
	(100; 200)	0.000	0.006	−0.413	0.000	
Azadirachta indica seed HAE	(0; 5)	0.038	0.000	−9.951	100.000	0.69
	(5; 12.5)	−0.021	0.570	−7.099	55.000	
	(12.5; 25)	−0.002	0.099	−2.080	25.000	
	(25; 50)	0.000	0.013	−0.676	10.000	
	(50; 100)	0.000	0.006	−0.186	0.000	
	(100; 200)	0.000	−0.001	0.068	0.000	
Garcinia lucida bark HAE	(0; 5)	−0.017	0.000	−0.584	100.000	0.85
	(5; 12.5)	0.018	−0.249	−1.831	95.000	
	(12.5; 25)	−0.005	0.165	−2.468	75.000	
	(25; 50)	0.001	−0.025	−0.721	60.000	
	(50; 100)	0.000	0.016	−0.937	35.000	
	(100; 200)	0.000	0.000	−0.137	15.000	

response, which occurs as a result of stress or infection, and leads to the larvae changing from cream-colored to dark brown or black. The two types of melanization in our study (Fig. 3B and C) can be explained by the fact that the high concentrations of the extracts were poorly distributed within the larvae unlike the low concentrations.

In addition, similarly to our study, seeds of *A. indica* have been reported to have high toxicity both in animal models (Braga *et al.*, 2021) and in insects (Islas *et al.*, 2020) due to phytochemicals that they contain, including azadirachtin, which is used in the composition of certain natural insecticides (Tofel *et al.*, 2017). This substance acts mainly by intercepting metamorphosis from the larval to adult stage and paralyzes the digestive tract of the larva (Tofel *et al.*, 2017; Meisyara *et al.*, 2019). Azadirachtin has been reported to be slightly soluble in water and freely soluble in polar organic solvents such as methanol, ethanol and ethyl acetate (Kale *et al.*, 2020), which could explain the large difference between the LD_{50}s of the. aqueous extract and the ethanolic extract of *A. indica* seed which were respectively 19.01 and 6.01 mg/ml. However, both ethanolic and aqueous extracts from the leaves of *A. indica* have demonstrated more than 10 times less toxicity than the seeds, possibly due to their different phytoconstituent composition.

According to the classification of Gosselin, Smith and Hodge (CCOHS, 2021), biologically active compounds can be super toxic (LD_{50} <5 mg/kg bw), extremely toxic [LD_{50} ϵ (5–50 mg/kg bw)], very toxic [LD_{50} ϵ (50–500 mg/kg bw)], moderately toxic [LD_{50} ϵ (0.5–5 g/kg bw)], slightly toxic [LD_{50} ϵ (5–15 g/kg)], and practically non-toxic (above 15 g/kg). Therefore, the data from our study indicate that ethanolic extract of *C. officinalis* bark and *A. indica* seed are both moderately toxic, *C. officinalis* aqueous bark extract and *G. lucida* ethanolic seed extract are slightly toxic while all the other plant materials extracts are practically non-toxic. Notwithstanding that the results of this study corroborate with some of those previously reported (for the same plants on vertebrate models), further investigations are

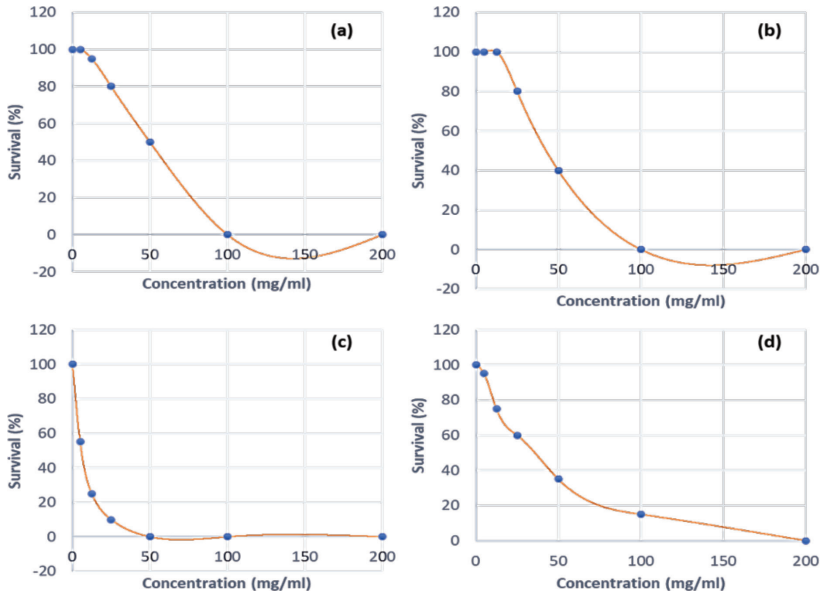

Fig. 2. Survival curves for *GM* larvae against (a): *Enantia chlorantha* ethanolic bark extract (HAE), (b): *Cymbopogon citratus leaves* HAE, (c): *Azadirachta indica* seed HAE (d): *Garcinia lucida bark* HAE. Each data point represents the mean percentage survival of 3 groups of 20 larvae, following injection with 20 µl of specific concentrations of the selected plant extract and incubation for 24 hours at 37°C.

Table 3. LD_{50}, LD_{90} and LD_{100} values for the medicinal plants tested on *GM* model.

Medicinal plants	Parts used	Extract	LD_{50}		LD_{90}		$\geq LD_{100}$	
			(mg/ml	g/kg of bw	mg/ml	g/kg of bw	mg/ml	g/kg of bw
Cinchona officinalis	Bark	EtOH	4.87	3.90	42.30	38.45	45.00	40.91
		H_20	9.53	8.66	50.00	38.46	98.03	75.41
Vernonia amygdalina	Leaves	EtOH	119.60	92.00	>200	>148.15	>200	>148.15
		H_20	>200	>148.15	>200	>173.91	>200	>173.91
Garcinia lucida	Seed	EtOH	14.93	12.98	66.64	51.26	100.00	76.92
		H_20	30.73	23.64	72.09	65.54	167.45	128.81
	Bark	EtOH	34.30	31.18	133.37	102.59	200.00	148.15
		H_20	64.22	49.40	184.21	141.70	>200	>173.91
Enantia chlorantha	Bark	EtOH	50.00	37.04	85.49	71.24	100.00	74.07
		H_20	100.00	86.96	200.00	160.00	>200	>173.91
Azadirachta indica	Leaves	EtOH	120.65	92.81	>200	>181.82	>200	>153.85
		H_20	>200	>166.67	>200	>153.85	>200	>160.00
	Seed	EtOH	6.01	4.81	25.00	18.52	50.00	45.45
		H_20	19.23	17.48	65.88	59.89	100.00	76.92
Moringa oleifera	Leaves	EtOH	55.79	42.92	87.30	67.15	100.00	74.07
		H_20	165.40	122.52	>200	>148.15	>200	>173.91
Cymbopogon citratus	Leaves	EtOH	41.14	35.77	81.03	70.46	100.00	76.92
		H_20	149.08	114.68	>200	>153.85	>200	>181.82

Fig. 3. (A): Healthy *GM* larva. (B): Larva showing slight and uniform melanisation. (C): Larvae showing localised melanization following injection with a LD_{100} of *C. officinalis* ethanolic bark extract.

required under the same experimental conditions (in terms of preparation of the extracts) to determine the correlation between the LD_{50}s in animal models and in *GM*. Finally, given the large number of larvae used in this study (1,100 larvae), this model may be of great utility if it is standardized as a study model for toxicity and may avoid animal sacrifices.

Conclusion

The data presented in this study indicate that the aqueous and ethanolic extracts of barks from *C. officinalis* as well as the ethanolic extract of seeds from *A. indica and G. lucida* induced a high toxic effect on *GM* larvae; while other plant material such as water extract of *M. oleifera*, *C. citratus*, and *V. amygdalina* leaves exerted low toxicity. The high variability of the LD values from one plant to another demonstrates the good sensitivity of *GM* and allows us to conclude that *GM* can be used as a reliable system model for the assessment of the toxicity of medicinal plants. However, further studies are needed to establish the exact correlation between LD_{50}, LD_{90}, LD_{100} values in *G. melonella* and in vertebrate models in order to facilitate reliable and precise extrapolations in humans.

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Conflict of interest

The authors declare that they have no competing interests.

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