

# Toxicity in *Artemia Salina* by Hydroalcoholic Extracts of Monocotyledonous and Dicotyledonous Varieties of Medicinal Plants from the Peruvian Amazon

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Medicinal plants have been used since ancient times, acquiring broad interest in their healing properties due to their active compounds. The Peruvian Amazon rainforest has a great variety of flora, such as monocots (*Dracontium lorentense* Krause and *Commelina diffusa*) and dicotyledons (*Dysphania ambrosioides*, *Malva sylvestris*, *Origanum vulgare*, *Bixa orellana*, *Pinus edulis*, *Jatropha curcas* L, and *Brunfelsia*). The study aimed to evaluate the toxicity in saline *artemia* by hydroalcoholic extracts of medicinal plants of the Peruvian Amazon. A phytochemical analysis of the leaves of the medicinal plants was performed to identify their active components. For the hydroalcoholic extraction, 300 g of leaves were used and dried extracts were obtained at concentrations of 10, 100 and 1000 µL/mL. The toxicity of the extract of each plant species in 10 larvae of *Artemia* was evaluated by triplicate tests. It was observed that chemical compounds such as steroids, triterpenes, quinones, phenolic compounds, flavonoids, lactones, alkaloids, reducing sugars, tannins and saponins, are the causes of toxicity in *artemia salina*, showing that the higher the concentration of the extract, the higher the index of mortality.

## 1. Introduction

Medicinal plants have gained interest in their healing potential due to the presence of phytochemicals (Afsar et al., 2015; Bokhari and Khan, 2015). The use of plants as a therapeutic mechanism is based on popular culture. Today the consumption of synthetic medicines has grown with high costs and limited access for the population, opting for the use of medicinal plants to address diseases as part of primary health care (WHO, 2015; Teles and Costa, 2014). The use of botanical drugs and plant derivatives were valued at \$ 23.2 and 24.4 billion between 2013 and 2014 respectively. This total market is expected to reach \$ 25.6 billion in 2015 and almost \$ 35.4 billion in 2020 (BCC, 2017)

Although synthetic drugs are available in most countries and are efficient, there are still people who opt for traditional medicine. Therefore, research has shown that parts of the plant such as seeds, fruit, leaves and fruits have been used for disease control. (Moraia et al., 2019). Likewise, a phytochemical detection must be carried out to identify the bioactivity that each active compound possesses, since it will allow to identify its advantages and disadvantages.

The ethnobotanical use of plants is important because it allows research and through it to discover new therapeutic alternatives, however, due to the daily consumption of plants for medicinal purposes, it is necessary to study their toxicology to avoid side effects (Ullah et al, 2014).

The Peruvian Amazon rainforest has a great diversity of flora, including monocotyledons (*Dracontium lorentense* Krause and *Commelina diffusa*) and dicotyledons (*Dysphania ambrosioides*, *Malva sylvestris*, *Origanum vulgare*, *Bixa orellana*, *Pinus edulis*, *Jatropha curcas* L, *Brunfraia* L, *Brunfraia* L, *Brunfraia* L, *Brunfraia* L, *Brunfraia* L.) (MINAM, 2019); species that are used as an option to traditional medicine necessary

for its active compounds, such as steroids, phenolic compounds, flavonoids, terpenes, reducing sugars, lactones among others (Pereira et al, 2016). Research has shown that leaf extracts of some species are toxic for human consumption due to the combination of their active compounds (Leite et al, 2015; Paredes et al, 2018).

*Artemia salina* is a light brown-bodied shrimp of the Crustaceae family, it has a size of 1 to 7 mm. This species is cosmopolitan and they live in salt water at a temperature of 6°C to 35°C. It feeds on algae and bacteria. Its physiology was studied as a preliminary test for toxicological investigations having its advantage, reliable results, low cost, easy handling, minimum requirements for laboratory manipulation, and highly sensitive to a wide variety of toxic agents; This trial being a practical, sustainable and sustainable method for evaluating the pharmacological potential of synthetic and natural compounds measured through their toxicity in plants (Ávalos et al, 2014 and Andrade et al, 2014). The toxicity of plants in *Artemia salina* implies only life or death, and the absence of toxicity is a starting point that biological systems can tolerate the plant (Silva et al, 2015). Therefore, the objective of the study was to evaluate the toxicity in saline artemia by hydroalcoholic extracts of medicinal plants of the Peruvian Amazon.

## **2. Methods**

### **2.1 Vegetal material**

500 grams of plant samples of each species (*Dysphania ambrosioides*, *Commelina diffusa*, *Malva sylvestris*, *Dracontium lorentense* Krause, *Origanum vulgare*, *Bixa orellana*, *Pinus edulis*, *Jatropha curcas* L, *Brunfelsia grandiflora*) were used, which were collected by researchers in the Cacatachi district San Martin region, 295 m. above sea level and 12 km northern of Tarapoto (6 ° 29'40 "south latitude and 76 ° 27'57" west longitude), Peru. The samples were placed in vacuum bags that were labeled with their names at a temperature of 37 °C, subsequently taken to the Truxillense Herbarium of the National University of Trujillo for identification and deposit with a registration code for each species: *Dysphania ambrosioides* (COD. 59598), *Commelina diffusa* (COD. 59600), *Malva sylvestris* (COD. 59601), *Dracontium lorentense* Krause (COD. 59602), *Origanum vulgare* (COD. 59604), *Bixa orellana* (COD. 59605), *Pinus edulis* (COD. 59606), *Jatropha curcas* L (COD. 59607), *Brunfelsia grandiflora* (COD. 59610).

### **2.2 Preparation of hydroalcoholic extract**

The leaves were washed with distilled water and disinfected with 96% ethanol. They were fractionated to an approximate size of 3 mm. For the extraction of the hydroalcoholic extract, the method was used by maceration with 300 g of leaves and 500 mL of 96% ethanol for 15 days under stirring with a vertical rotary evaporator (Scilogex RE-100) at 70 revolutions per minute to obtain dry extracts. With the sample obtained, dilutions were prepared at concentrations of 10, 100 and 1000 µg/mL.

### **2.3 Phytochemical analysis**

The hydroalcoholic leaf extract was evaluated with the purpose of identifying its active ingredients such as: steroids, triterpenes, quinones, phenolic compounds, flavonoids, lactones, alkaloids, reducing sugars, tannins and saponins, using the protocol described by Lock (2016). 1 mL of pure extract was added to 10 test tubes to see the metabolites in each hydroalcoholic extract, and tests were performed to identify secondary metabolites through color change, being classified as light, moderate or strong. The tests used to determine the presence of each type of secondary metabolite are listed in Table 1.

### **2.4 Obtaining and breeding *Artemia salina*.**

The eggs of *Artemia salina* 20 days old were provided by the Animal Physiology Department, National University of Trujillo, washed with filtered seawater to remove impurities and were transported to a glass incubation chamber with abundant oxygenation was used one gram of eggs (equivalent to 700-800 eggs), allowing to incubate in 5 liters of filtered seawater under artificial fluorescent light at 110 Watts, temperature of 25 °C and adjusted to a pH of 7-8 for 24 hours. *Artemia salina* eggs were fed commercial yeast extract to hatch and continue their biological cycle for approximately seven days. Then 10 larvae were used for each concentration of 7 old days (10, 100 and 1000 µg / mL) in stage III as toxicity markers due to their high sensitivity (Silva et al, 2015; Ravi et al, 2014; Jaramillo et al, 2016).

Table 1. Phytochemical analysis of the hydroalcoholic extract of the medicinal plants leaves from the Peruvian Amazon

Test	Secondary Metabolites	Monocotyledons				Dicotyledons				
		<i>Commelina diffusa</i>	<i>Dracontium loretense</i> L	<i>Brunfelsia grandiflora</i>	<i>Malva sylvestris</i>	<i>Dysphania ambrosioides</i>	<i>Pinus edulis</i>	<i>Bixa Orellana</i>	<i>Jatropha curcas</i> L	<i>Origanum vulgare</i>
Liebermann-Bouchard	Steroids and triterpens	++	++	+	+	++	+	++	++	++
Borntrager	Quinones	-	-	-	-	-	-	-	-	-
ferric Chloride	phenolic Compounds	+++	++	+	++	++	+	+	++	++
Shinoda	Flavonoids	++	++	-	++	++	-	++	++	+++
Baljet	lactones	-	-	+	-	+	-	+	-	-
Dragendorff	Alkaloids	-	-	-	-	-	-	-	-	-
Mayer	Alkaloids	-	-	-	-	-	-	-	-	-
Fehling	reducing Sugars	++	++	+	+	-	-	++	+	+
Gelatine Foam	tannins	-	-	-	-	-	-	+	-	-
	Saponins	-	-	+	-	-	-	-	-	-

Note: Color changes of secondary metabolites in (+) = Light, (++) = moderate and (+++) = strong

## 2.5 Toxicity tests

The concentrations of 10, 100 and 1000 µg/mL of filtered seawater were prepared according to the protocol described by Seremet et al (2018). Then 5 µg of extract was diluted in 5 mL of filtered seawater, equivalent to 10 µg/mL, 50 µg of extract in 5 mL of filtered seawater, equivalent to 100 µg/mL, and 500 µg of extract in 5 mL of filtered seawater, equivalent to 1000 µg/mL. The larvae were placed in a test tube containing 10 mL of filtered seawater and 0.5 mL of the hydroalcoholic extract. 10 larvae were used for each plant species and concentration; each test was performed in triplicate. A control group of 10 larvae in 10 mL of filtered seawater without extract was used to make the respective comparisons. The analyses were performed in the biological chemistry laboratory of the National University of Trujillo, the larvae were exposed to the treatments for 24 hours, after this time the number of larvae was considered dead only if there was no movement of their appendages for 10 seconds (Socea et al, 2015). For this, a stereoscope was used (Eurolab NSD-405). The toxicity criteria for *Artemia salina* samples were classified as follows: > 1000 µg/mL (non-toxic), 500 DL 50 ≤ 1000 (low toxicity), 100 < DL 50 ≤ 500 (moderate toxicity), DL 50 < 100 (high toxicity) (Arana et al, 2016; Alonso et al, 2017 and Monteiro et al, 2018). The organisms toxicity percentage exposed to the effect of the extracts was estimated as follows:

$$\text{Toxicity (percentage)} = (\text{TNA} - \text{NAA}) / \text{TNA} * 100 \quad (1)$$

Where: TNA = Total number of *Artemia*

NAA = Number of *Artemia* alive (Jan and Khan, 2016)

## 2.6 Ethical statement

*Artemia salina* does not represent a danger to the environment, it is not an endangered species, because it does not appear on the red list of the International Union for the Conservation of Nature (IUCN), the species is used for scientific purposes (IUCN, 2019).

## 3. Results

In Figure 1, it is observed that of 9 species of medicinal plants only 7 have high toxicity (HT), being at least 56.7% death in *Artemia salina* caused by *Bixa Orellana* and the maximum percentage 86.7% caused by *Commelina diffusa*.

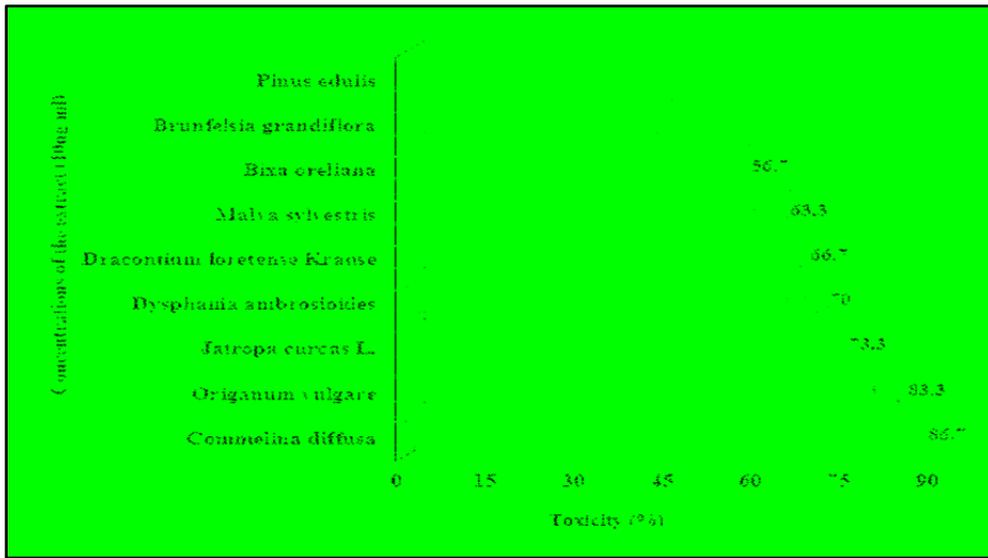


Figure 1. Percentage of toxicity of 10 µg/mL of hydroalcoholic extract of medicinal plants.

In Figure 2, it is observed that of 9 species of medicinal plants only 6 have moderate toxicity (MT), being at least 53.3% of death in *Artemia salina* is caused by *Dracontium lorentense Krause* and the maximum percentage 66.7% is by *Commelina diffusa*.

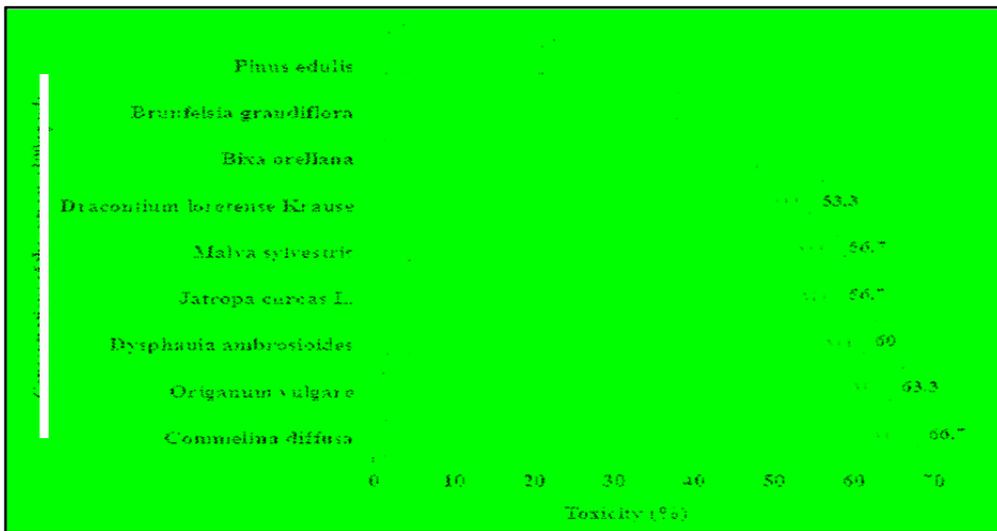


Figure 2. Percentage of toxicity of 100 µg/mL of hydroalcoholic extract of medicinal plants.

In Figure 3, it is observed that the 9 species of medicinal plants have low toxicity (LT), finding that the minimum percentage 17% of death in *Artemia salina* is caused by *Pinus edulis* and the maximum percentage 43.3% is by *Origanum vulgare*.

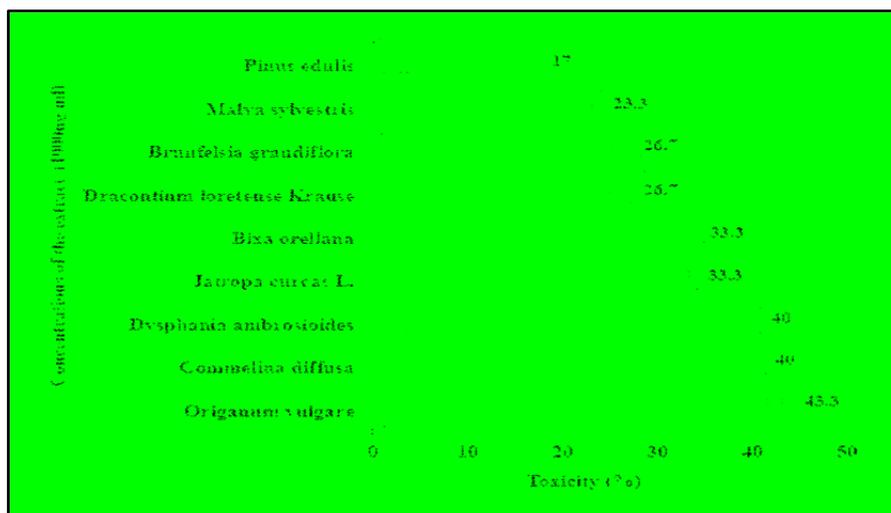


Figure 3. Percentage of toxicity of 1000 µg / mL of hydroalcoholic extract of medicinal plants.

#### 4. Conclusions

If the concentration is greater than 10 µg/mL, toxicity is high in hydroalcoholic extracts of 7 plant species in *Artemia salina*. Also, if the concentration is less than 1000 µg/mL, toxicity is low in all hydroalcoholic extracts. The consumption of medicinal plants has been increasing due to its probable effectiveness. However, its indiscriminate use is a latent risk due to the toxicity of some compounds within the plant that can cause collateral damage. For this reason, it may be useful to study extracts of medicinal plants in order to show therapeutic or toxic activity of their active compounds using *Artemia salina* as an evaluation procedure (Simões and Almeida, 2015).

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