



Lethality of Hydroalcoholic Extracts from Fruit Plant Leaves in the Peruvian Jungle

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Lethality of hydroalcoholic extract from fruit plant leaves in the Peruvian jungle in *Artemia salina* was evaluated. The type of study was experimental with a control group ($K_2Cr_2O_7$). The biological material was fruit leaves (*Cocos nucifera*, *Mauritia flexuosa*, *Theobroma cacao* L., *Coffea* sp, and *Musa* sp) collected in San Martín Region; Likewise, the phytochemical march to the leaves was carried out to identify their active principles. The *Artemia salina* eggs were provided by the department of animal physiology of the National University of Trujillo, they were kept under specific conditions such as artificial light, a temperature of 25 ° C and a time of 24 hours, allowing them to mature up to 48 hours. For the preparation of the hydroalcoholic extract it was by the maceration method using 500g of leaves and 500mL of 70° alcohol; for 15 days under stirring, the solution was taken to a vertical rotary evaporator to obtain a dry extract preparing concentrations of 10, 100, 250, 500 and 1000 µg/mL. The sample consisted of 10 larvae for each plant species and concentration, performing the test in triplicate. The LC50 lethality of *Artemia salina* in the samples was classified as: >1000 µg/mL. (Non- lethality), 500 < LC50 ≤ 1000 (Low toxicity), 100 < LC50 ≤ 500 (Moderate lethality), LC50 < 100 (High lethality). It was obtained as a result that *Mauritia flexuosa* and *Theobroma cacao* L. in concentrations 10, 100 µg/mL present high and moderate lethality.

1. Introduction

Medicinal plants have been used since ancient times as a curative means, receiving interest for their healing potential due to their active components (Afsar et al., 2015). Nowadays, the consumption of medicines has raised their costs which has generated limited access for the population, opting for the use of medicinal plants for the treatment of diseases as part of primary health care (WHO, 2015; Teles and Costa, 2014). The use of botanical and plant-derived medicines was valued at \$23.2 and \$24.4 million between 2013 and 2014 and is expected to reach \$25.6 million in 2015 and \$35.4 billion in 2020 (BCC, 2017). The ethnobotanical use of plants is important because it allows research and through it the discovery of new therapeutic alternatives. Studies have shown that plant parts such as seeds, fruits, leaves and roots have been used for disease control (Moraia et al., 2019). However, a phytochemical analysis must be performed to identify the bioactivity possessed by each active compound to avoid side effects (Ullah et al., 2014).

The Peruvian rainforest has a great diversity of flora including fruit plants of species *Cocos nucifera*, *Mauritia flexuosa*, *Theobroma cacao* L., *Coffea* sp and *Musa* sp (MINAM, 2019); species that are used as an alternative for traditional medicine due to their active principles, such as steroids, phenolic compounds, flavonoids, terpenes, reducing sugars, lactones, among others (Pereira et al., 2016; Sandoval et al., 2020). Some research has shown that hydroalcoholic extracts of leaves of some species are toxic for human consumption due to the combination of their active compounds (Leite et al, 2015; Paredes et al., 2018; Sandoval et al., 2020). *Artemia salina* is a light brown shrimp of the Crustaceae family, with a size of 1 to 7 mm. It is cosmopolitan, lives in salt water at a temperature of 6°C to 35°C, and feeds on algae and bacteria. The study of its physiology concludes in performing preliminary tests because they are low cost, easy to handle and present minimum requirements for laboratory manipulation. Toxicology studies indicate the presence of sensitivity to certain toxic agents and

therefore provide reliable results. As this is a practical, sustainable and sustainable method, it is used to evaluate the pharmacological potential of synthetic and natural compounds measured through their lethality in plants, which implies only life or death (Avalos et al., 2014; Silva et al., 2015). Therefore, the objective of the study was to evaluate the lethality of hydroalcoholic extracts of Peruvian rainforest fruit leaves on *Artemia salina*.

2. Method

2.1. Vegetal material

500 grams of leaves of the species *Cocos nucifera*, *Mauritia flexuosa*, *Theobroma cacao L.*, *Coffea sp* and *Musa sp*, were collected in the district of Cacatachi, San Martín, at 295 m. above sea level and 12 km north of Tarapoto (6°29'40" south latitude and 76°27'57" west longitude). The samples were placed in vacuum bags and labeled with their name at a temperature of 37 °C. Afterwards, they were taken to the Truxillense Herbarium of the Universidad Nacional de Trujillo (National University of Trujillo) for identification and deposit with a registration code for each species: *Cocos nucifera* (COD. 59603), *Mauritia flexuosa* (COD.59597), *Theobroma cacao L.* (COD. 59599), *Coffea sp* (COD. 59609) and *Musa sp* (59608).

2.2 Preparation of hydroalcoholic extract

The leaves were washed with distilled water and disinfected with 70° ethanol. They were fractioned to an approximate size of 4 mm. For the extraction of the hydroalcoholic extract, the maceration method was used: 350 g of leaves and 500 mL with 70° ethanol for 15 days under agitation with a vertical rotary evaporator (Scilogex RE-100) at 75 revolutions per minute to obtain dry extracts. Dilutions at concentrations of 10, 100, 250, 500 and 1000 µg/mL were prepared with the sample obtained.

3.3 Phytochemical analysis

The hydroalcoholic extract of fruit leaves was evaluated in order to identify its active principles. Each sample was subjected to solvents of increasing polarity to obtain secondary metabolites according to their solubility using reagents and dyes to determine the presence or absence of active components such as: steroids, triterpenes, quinones, phenolic compounds, flavonoids, lactones, alkaloids, reducing sugars, tannins and saponins, by using the protocol described by Lock (2016). 3 mL of pure extract was added to 10 test tubes to identify secondary metabolites through color change, classified as light, moderate or strong. The tests used to determine the presence of each type of secondary metabolite are listed in Table 1.

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

Assay	Secondary metabolites	<i>Cocos nucifera</i>	<i>Coffea sp</i>	<i>Theobroma cacao L</i>	<i>Musa sp</i>	<i>Mauritia flexuosa</i>
Lieberman-Bouchard	Steroids and triterpenes	(++)	(+)	(++)	(++)	(++)
Borntrager	Quinones	(-)	(-)	(-)	(-)	(-)
Ferric chloride	Phenolic compounds	(+)	(+)	(+)	(++)	(+++)
Shinoda	Flavonoids	(+)	(+)	(-)	(+++)	(+++)
Baljet	Lactones	(+)	(-)	(-)	(++)	(+)
Dragendorff	Alkaloids	(-)	(+)	(-)	(-)	(-)
Mayer	Alkaloids	(-)	(+)	(-)	(-)	(-)
Fehling	Sugar reducers	(++)	(+)	(++)	(++)	(++)
Gelatin	Tannins	(++)	(+)	(-)	(-)	(++)
Foam	Saponins	(-)	(-)	(-)	(-)	(-)

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

2.4 Obtaining and breeding *Artemia salina*

The 20-day-old *Artemia salina* eggs were provided by the Department of Animal Physiology, Universidad Nacional de Trujillo, and were washed with filtered seawater to remove impurities. An incubation chamber with abundant oxygenation was used, adding to this container one gram of eggs (equivalent to 700-800 eggs), allowing incubation 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 h. The *Artemia salina* eggs were fed with commercial yeast extract to hatch and continue their biological cycle for approximately seven days. Then, 10 7-day-old larvae per each

concentration (10, 100, 250, 500 and 1000 µg/mL) at stage III were used as a toxicity marker due to its high sensitivity (Silva et al., 2015; Jaramillo et al., 2016).

2.5 Lethality testing

Concentrations of 10, 100, 250, 500 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, 125 µg of extract in 5 mL of filtered seawater, equivalent to 250 µg/mL, 250 µg of extract in 5 mL of filtered seawater, equivalent to 500 µg/mL and 500 µg of extract in 5 mL of filtered seawater, equivalent to 1000 µg/mL. Ten larvae were placed in a test tube containing 10 mL of filtered seawater and 0.5 mL of the hydroalcoholic extract; this was for each plant species and concentration. Each assay was performed in triplicate and a K₂Cr₂O₇ control group of 250 µg/mL was used for comparison (Goncalves et al., 2019; Simoes and Almeida, 2015). The larvae were exposed to the treatments for 24 hours; after this time the number of dead larvae was counted only if there was no movement of their appendages for 10 seconds (Socea et al., 2015); for this purpose, a stereoscope (Eurolab NSD-405) was used.

The toxicity criteria for *Artemia salina* samples were classified as follows: > 1000 µg/mL (non-toxic), 500 LD 50 ≤ 1000 (low toxicity), 100 < LD 50 ≤ 500 (moderate toxicity), LD 50 < 100 (high toxicity) (Alonso et al., 2017 and Monteiro et al., 2018). The toxicity percentage of the organisms exposed to the effect of the extract was estimated as follows:

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

Where: TNA = Total number of *Artemia salina*.

NAA = Number of live *Artemia salina* (Jan and Khan, 2016).

2.6 Ethical Statement

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

3. Results

It is observed that the 5 plant species have high lethality compared to the positive control group, with *Mauritia flexuosa* having the highest percentage of lethality.

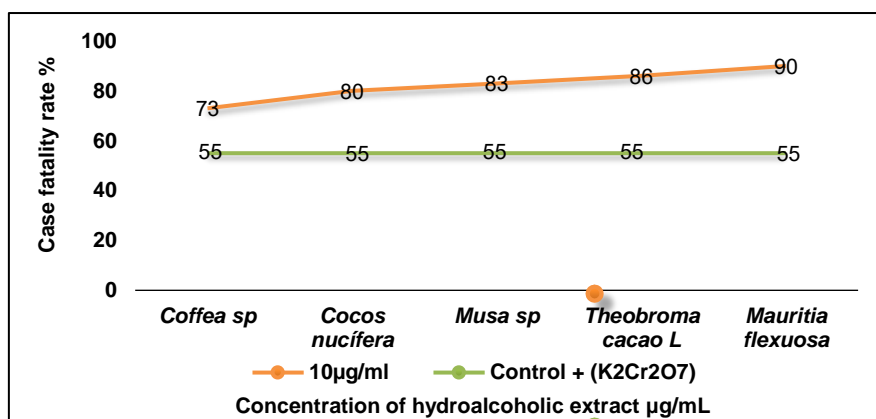


Figure 1. Concentration of 10µg/mL of hydroalcoholic extract vs. positive control group (K₂Cr₂O₇).

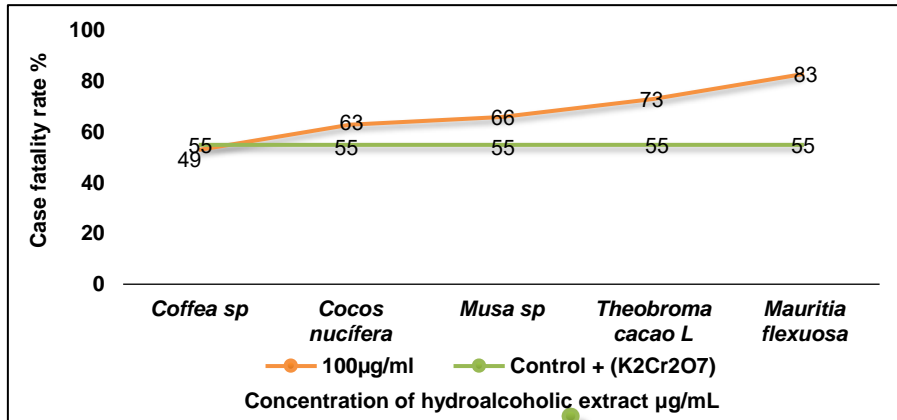


Figure 2. Concentration of 100µg/mL of hydroalcoholic extract vs. positive control group (K₂Cr₂O₇).

It is evident that the 4 plant species have high lethality compared to the positive control group, with *Mauritia flexuosa* having the highest percentage of lethality.

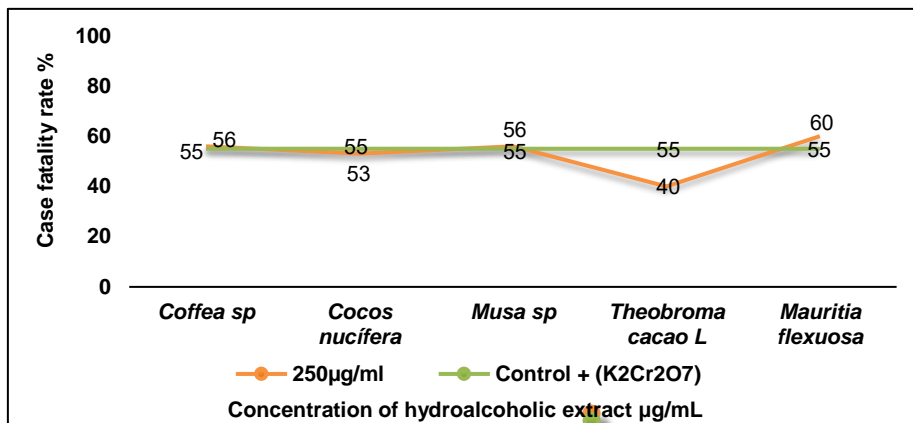


Figure 3. Concentration of 250µg/mL of hydroalcoholic extract vs. positive control group (K₂Cr₂O₇).

It is observed that the 4 plant species have moderate lethality (*Coffea sp*, *Cocos nucifera*, *Musa sp* and *Mauritia flexuosa*) compared to the positive control group, with *Mauritia flexuosa* having the highest percentage of lethality.

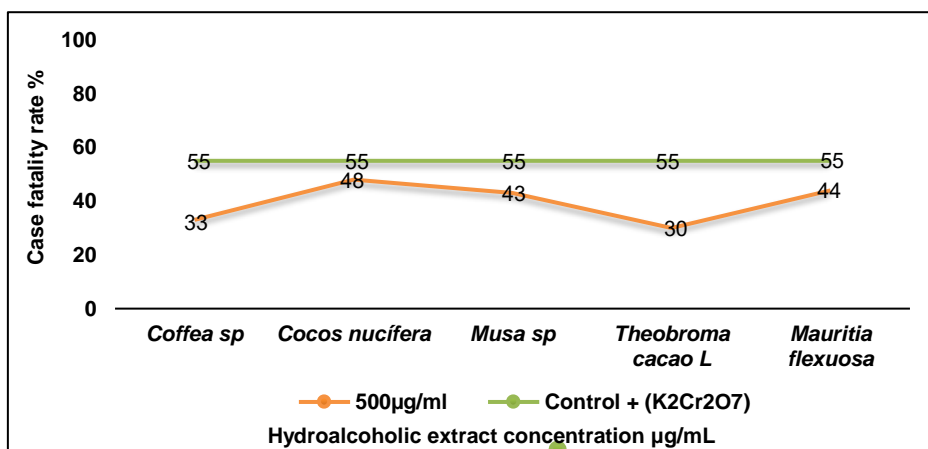


Figure 4. Concentration of 500µg/mL of hydroalcoholic extract vs. positive control group (K₂Cr₂O₇).

It is evident that the 5 plant species have low lethality compared to the positive control group, which has the highest percentage of lethality.

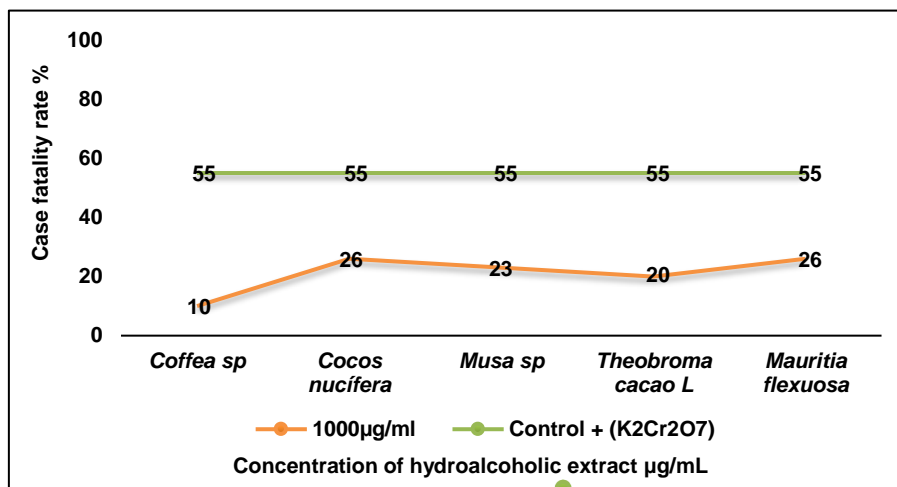


Figure 5. Concentration of 1000 µg/mL of hydroalcoholic extract vs. positive control group (K₂Cr₂O₇).

It is observed that the 5 plant species do not present lethality compared to the positive control group, which presents the highest percentage of lethality.

4. Conclusions

The active compounds of the hydroalcoholic extracts of fruit leaves from the Peruvian rainforest were analyzed, finding steroids, triterpenes, phenolic compounds, flavonoids and tannins. *Artemia salina* was used as an indicator to measure lethality. The results indicated that concentrations less than or equal to 100 µg/mL have high lethality, concentrations of 250 µg/mL moderate lethality, concentrations of 500 µg/mL low lethality and concentrations equal to or greater than 1000 µg/mL are not lethal. The hydroalcoholic extracts with the highest lethality concentration were *Mauritia flexuosa* and *Theobroma cacao L*. The consumption of medicinal plants has been increasing due to their probable effectiveness. However, 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 medicinal plant extracts to show therapeutic or toxic activity of their active compounds. Other studies indicate that if a sample is not lethal to *Artemia salina*, its effects will also be similar for humans.

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