RESEARCH NOTE

Toxicity of the hydroalcoholic extracts of fruit leaves from the Peruvian Amazon in *Artemia salina* [version 1; peer review: 1 approved with reservations]

Ana Sandoval Vergara ¹, Armando Ismiño Riquelme²

¹Universidad César Vallejo, Carretera Marginal Norte Fernando Belaúnde Terry Km. 8.5, Sector Maronilla Mishquiyacu, Cacatachi, San Martín, Peru

²Asociación de Productores Jardines de Palma, Pongo de Caynarachi, San Martín, Peru

V1 First published: 08 Jul 2019, 8:1016
<https://doi.org/10.12688/f1000research.18997.1>

Latest published: 08 Jul 2019, 8:1016
<https://doi.org/10.12688/f1000research.18997.1>

Abstract

Background: Medicinal plants have been used since ancient times, receiving interest in their healing potential because of their active components. The Amazon rainforest in the east of Peru has great diversity of flora, especially monocotyledons (*Cocos nucifera*, *Mauritia flexuosa* and *Coffea* spp.) and dicotyledons (*Theobroma cacao* L. and *Musa* spp). The toxicity of the hydroalcoholic extract of plant leaves from the Peruvian Amazon in *Artemia salina* was evaluated in this study.

Methods: The leaves of the plants *Cocos nucifera*, *Mauritia flexuosa*, *Theobroma cacao* L., *Coffea* sp, and *Musa* sp were collected in the district of Cacatachi, San Martín. Phytochemical analysis of the leaves was carried out to identify their active components. The eggs of *Artemia salina* were provided by the Department of Animal Physiology of Universidad Nacional de Trujillo. The hydro-alcoholic extraction was carried out via the maceration method using 300 g of leaves and 500 mL of 96% ethanol for 15 days in agitation. The solutions were taken to a vertical rotavapor to obtain dry extracts and concentrations of 10, 100 and 1000 µL/ml were prepared. To test toxicity, 10 larvae were given the extract for each plant species and concentration in triplicate. The CL₅₀ toxicity of *Artemia salina* samples was classified as: > 1000 µL/ml (non-toxic), 500<CL₅₀ ≤ 1000 (low toxicity), 100<CL₅₀ ≤ 500 (moderate toxicity), CL₅₀<100 (high toxicity).

Results: It was observed that *Mauritia flexuosa* and *Musa* sp. at concentrations of 10, 100 and 1000 µL/ml have high, medium and low toxicity, respectively. However, only low toxicity was observed in *Cocos nucifera*, *Theobroma cacao* L. and *Coffea* sp.

Conclusions: It can be concluded that the obtained results are in accordance with other studies that examined different extracts, indicating that if a sample is non-toxic to *Artemia salina*, then its effects will also be similar in humans.

Peer review discontinued

Peer review at F1000Research is author-driven. Currently no reviewers are being invited. [What does this mean?](#)

Approval Status ?

	1
version 1	view
08 Jul 2019	

1. **Gabriel Alfonso Gutierrez-Rebolledo** ,
 National Polytechnic Institute, Mexico City,
 Mexico

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

Toxicity, hydro-alcoholic extract, Peruvian Amazon, Artemia salina

Corresponding author: Ana Sandoval Vergara (asandoval@ucv.edu.pe)

Author roles: **Sandoval Vergara A:** Conceptualization, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Ismiño Riquelme A:** Investigation, Methodology, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2019 Sandoval Vergara A and Ismiño Riquelme A. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Sandoval Vergara A and Ismiño Riquelme A. **Toxicity of the hydroalcoholic extracts of fruit leaves from the Peruvian Amazon in *Artemia salina* [version 1; peer review: 1 approved with reservations]** F1000Research 2019, 8:1016 <https://doi.org/10.12688/f1000research.18997.1>

First published: 08 Jul 2019, 8:1016 <https://doi.org/10.12688/f1000research.18997.1>

Introduction

Medicinal plants have been used since ancient times, receiving interest in their healing potential because of their active components^{1,2}. Some developing countries use plants in primary health care, and in 2015 it was estimated that 25.6 billion dollars were spent worldwide on plant consumption and its derivatives, expected to increase to 35.4 billion dollars by 2020^{3,4}. Due to the high consumption of plants for medicinal purposes, it is necessary to study their toxicology in order to avoid side effects.

The Amazon rainforest in the east of Peru has great diversity of flora, especially monocotyledons (*Cocos nucifera*, *Mauritia flexuosa* and *Coffea* spp.) and dicotyledons (*Theobroma cacao* L. and *Musa* spp.); species which are used as an alternative to medicine due to their chemical properties and active components, such as steroids, phenolic compounds, flavonoids and lactones⁵⁻⁷. Studies have shown that the leaf extracts of some species are toxic for the human consumption because of the combination of their chemical compounds⁸⁻¹¹.

Artemia salina is a species of shrimp belonging to the Crustacea family. Ranging from 1 to 7 mm in size, these shrimps have a cosmopolitan distribution and live in saltwater at a temperature of 6 to 35°C¹². Its biology was studied as a preliminary test for ecotoxicological investigations associated with low cost and easy handling, in addition to its potential use as a practical and sustainable method for the identification of the pharmacological potential of synthetic and natural compounds and to measure their toxicity in animals¹³⁻¹⁵. The determination of plant toxicity against *Artemia salina* involves only one parameter: life or death, and the absence of toxicity is an indicator that the plant can be tolerated by biological systems¹⁶. Therefore, the aim of this study was to evaluate the toxicity of the hydro-alcoholic extract of fruit leaves from the Peruvian Amazon in *Artemia salina*.

Methods

Source and husbandry of *Artemia salina*

The eggs (20 days old) of *Artemia salina* were donated by the Department of Animal Physiology of Universidad Nacional de Trujillo, washed with filtered seawater and dried in dehydrated environment and then transported (with oxygen supply).

One gram of eggs was used (equivalent to 700–800 eggs), left to hatch in 5 liters of filtered sea water under fluorescent artificial light (110 watts) at a temperature of 25°C for 24 h. The eggs were fed on commercial yeast extract and kept in a glass incubation chamber with abundant oxygenation to allow them to hatch and continue their biological cycle for approximately seven days. Stage III larvae (seven days old) were used as toxicity markers due to their high sensitivity and 10 larvae were used to test each concentration (10, 100 and 1000 µg / ml).

Source of plant samples

A total of 500 grams of plant samples from each species (*Cocos nucifera*, *Mauritia flexuosa*, *Theobroma cacao* L., *Coffea* sp. and *Musa* sp) were collected by researchers (AI and AS) in the district of Cacatachi, San Martín at 295 m above sea level and 12 km to the north of Tarapoto (6°29'40" south latitude and 76°27'57" west longitude). The specimens were transferred in vacuum bags that were labeled with their respective scientific names at a temperature of 37°C to the Herbarium Truxillense of Universidad Nacional de Trujillo for their identification and given a registration code.

Preparation of plant samples

Leaves were washed with distilled water, disinfected with 96% ethanol and fragmented to an approximate size of 3mm. Extraction of the hydro-alcoholic extract was carried out by maceration with 300 g of leaves and 500 mL of 96% ethanol over 15 days, with agitation carried out using a vertical rotavapor (Scilogex RE-100), run at 70rpm for 10 minutes every four hours (between 7am and 10pm) and a dry extract dissolved in 96% ethanol was obtained.

Phytochemical analysis of plant samples

Phytochemical analysis of the leaf samples was carried out to identify their active principles according to the protocol described by Miranda *et al.*¹⁷. Briefly, each sample was subjected to solvents of increasing polarity, in order to obtain secondary metabolites according to their solubility, using reagents and dyes to determine the presence or absence of active components such as terpenes, flavonoids, reducing sugars, among others. The assays used to determine the presence of each type of secondary metabolite are listed in Table 1. To each test tube,

Table 1. Phytochemical analysis of the fruit leaves of the Peruvian Amazon.

Assay	Metabolites	Mauritia flexuosa	Cocos nucifera	Theobroma cacao L.	Coffea sp	Musa sp
Lieberman-Bouchard	Steroids and Triterpenes	(++)	(++)	(++)	(+)	(++)
Ferric chloride	Phenolic compounds	(+++)	(+)	(+)	(+)	(++)
Shinoda	Flavonoids	(+++)	(+)	-	(+)	(+++)
Baljet	Lactones	(+)	(+)	-	-	(++)
Dragendorff	Alkaloids	-	-	-	(+)	-
Mayer	Alkaloids	-	-	-	(+)	-
Fehling	Reducing sugars	(++)	(++)	(++)	(+)	(++)
Gelatine	Tannins	(++)	(++)	-	(+)	-

(+): light, (++): moderate, (+++): strong.

1ml of pure extract was added and reagents for each assay were added that identify secondary metabolites through color change. The results of the color change were judged by eye according to the method described by Miranda and Cuellar¹⁷ and classified as light, moderate or strong.

Toxicity tests

Concentrations (using filtered seawater) of 10, 100 and 1000 µg/ml were then prepared according to the protocol described by Seremet *et al.* 2018¹⁸: 5µg of extract was diluted in 5ml of filtered seawater, equivalent to 10µg/ml, 50µg of extract was diluted in 5ml of filtered seawater, equivalent to 100µg/ml, and 500µg of extract was diluted in 5ml of filtered seawater, equivalent to 1000µg/ml. The larvae were added to a test tube containing 10ml of filtered seawater and 0.5ml of the hydroalcoholic extract. A total of 10 larvae were used for each plant species and concentration and each test was performed in triplicate. A control group of 10 larvae in 10ml of filtered seawater without extract was used. The tests were performed in the biological chemistry laboratory of the Universidad Nacional de Trujillo and larvae were observed for 24 hours before the number of live larvae were counted using a stereoscope. The lethal concentration (CL₅₀) for *Artemia salina* samples was used to classify toxicity as follows: > 1000 µg/ml (non-toxic), 500 < CL₅₀ ≤ 1000 (low toxicity), 100 < CL₅₀ ≤ 500 (moderate toxicity), CL₅₀ < 100 (high toxicity)¹⁹.

The toxicity percentage was calculated as follows:

$$\text{Toxicity(percent)} = (TNA - AA) / TNA * 100$$

Where TNA = Total number of *Artemia* and AA = Number of alive *Artemia*¹⁹.

Statistical analysis

The statistical analysis was carried out using SPSS version 23, applying the student's t-test to calculate toxicity at different concentrations, with p<0.05 considered as statistically significant.

Ethical statement

Generally, permits are required from the Environmental Ministry to perform experiments in which animals are involved. However, a permit to investigate *Artemia salina* was not considered necessary because it does not appear on the [International Union for the Conservation of Nature \(IUCN\)](#) red list and therefore it's investigation does not represent a danger to the environment or to human beings. All efforts were made to ameliorate any suffering to the animals following the protocol described by the Ecuadorian code of practice for the care and use of animals for scientific purposes of the Ministry of Environment of Ecuador.

Results

Qualitative presence of phenolic compounds can be observed in the hydro-alcoholic extract, such as flavonoids, steroids, triterpenes, reducing sugars, lactones and tannins (Table 1)²⁰.

In Figure 1, it can be observed that there is high toxicity (10 µg/ml), moderate (100 µg/ml) and low (1000 µg/ml) in

Figure 1. Toxicity percentage at different concentrations of hydro-alcoholic extract.

Mauritia flexuosa and *Musa sp*; however, only low toxicity (1000 µg/ml) is evidenced in *Cocos nucifera*, *Coffea sp.* and *Theobroma cacao L* (see *Underlying data*)²¹.

The results obtained with *Artemia salina* indicate that both *Mauritia flexuosa* and *Musa sp* in the concentrations 10, 100 and 1000 µg/ml have low, moderate and high toxicity, respectively. However, in *Cocos nucifera*, *Coffea sp* and *Theobroma cacao L.*, only low toxicity is observed at a concentration of 1000 µg/ml, indicating that toxicity was directly proportional to the concentration of the extract used; therefore, the highest toxicity could be related to the high content of phenolic compounds and flavonoids, among other components, in accordance with previous studies^{22–26}. Samples with values of LD₅₀ higher than 1,000 µg/ml were considered non-toxic, in accordance with Leite *et al.* 2009²⁷, and values of LD₅₀ below 1,000 µg/ml were considered to be toxic according to previous studies^{28–30}. The characteristic phytochemical composition of each plant can change due to various factors such as room temperature, soil, pH and origin. These environmental conditions may influence the synthesis^{31,32} and expression of the phytochemical components in the plant, changing their toxicity.

Conclusions

Health treatments with medicinal plants are cheap and accessible to the population; however, their indiscriminate use is a risk due to the toxicity of some compounds within the plant. For this reason, it may be useful to study plant extracts with the aim of demonstrating the therapeutic or toxic action of their active components, using *Artemia salina* as an evaluation method. In this context, the toxicity of hydroalcoholic extracts of fruit leaves from the Peruvian Amazon was evaluated. It can be concluded that the obtained results are in accordance with other studies that examined different extracts as reported by Simões and De Almeida³³, indicating that if a sample is non-toxic to *Artemia salina*, then its effects will also be similar to humans.

Data availability

Underlying data

Figshare: Toxicity percentage at different concentrations of hydro-alcoholic extract. <https://doi.org/10.6084/m9.figshare.7996598.v2>²¹

This project contains the following underlying data:

- Toxicity table.xlsx (spreadsheet containing raw toxicity data)

Figshare: Photos for the phytochemical analysis of the fruit leaves. <https://doi.org/10.6084/m9.figshare.8194580.v2>²⁰

This project contains the following underlying data:

- Cocos nucifera.tif (photographs of the results of the phytochemical analysis for Cocos nucifera)
- Coffea Sp.tif (photograph of the results of the phytochemical analysis for Coffea Sp)
- Mauritia flexuosa.tif (photograph of the results of the phytochemical analysis for Mauritia flexuosa)
- Musa Sp.tif (photograph of the results of the phytochemical analysis for Musa Sp)
- Theobroma cacao L.tif (photograph of the results of the phytochemical analysis for Theobroma cacao L)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Grant information

The author(s) declared that no grants were involved in supporting this work.

References

1. Gouveia-Figueira SC, Gouveia CA, Carvalho MJ, *et al.*: **Antioxidant Capacity, Cytotoxicity and Antimycobacterial Activity of Madeira Archipelago Endemic *Helichrysum* Dietary and Medicinal Plants.** *Antioxidants (Basel)*. 2014; 3(4): 713–29.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
2. Ullah S, Rashid Khan M, Ali Shah N, *et al.*: **Ethnomedicinal plant use value in the Lakki Marwat District of Pakistan.** *J Ethnopharmacol*. 2014; 158 Pt A: 412–22.
[PubMed Abstract](#) | [Publisher Full Text](#)
3. Taddei-Bringas GA, Santillana-Macedo MA, Romero-Cancio JA, *et al.*: **[Acceptance and use of medicinal plants in family medicine].** *Salud Publica Mex*. 1999; 41(3): 216–220.
[PubMed Abstract](#)
4. **Fármacos botánicos y derivados de plantas. Mercados globales.** Informe de investigación BCC BIO022G.
[Reference Source](#)
5. Krishnaiah D, Sarbatly R, Nithyanandam R: **A review of the antioxidant potential of medicinal plant species.** *Food and Bioprocess Processing*. 2011; 89(3): 217–233.
[Publisher Full Text](#)
6. Pereira Freire JA, Barros KBNT, Lima LKF, *et al.*: **Phytochemistry Profile, Nutritional Properties and Pharmacological Activities of *Mauritia flexuosa*.** *J Food Sci*. 2016; 81(11): R2611–R2622.
[PubMed Abstract](#) | [Publisher Full Text](#)
7. Shah NA, Khan MR, Naz K, *et al.*: **Antioxidant potential, DNA protection, and HPLC-DAD analysis of neglected medicinal *Jurinea dolomiæa* roots.** *Biomed Res Int*. 2014; 2014: 726241.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Martínez M, Ocampo D, Galvis A, *et al.*: **Actividad antibacteriana y citotoxicidad in vitro de extractos etanólicos de *Bauhinia variegata* L. (Fabaceae).** *Revista Cubana de plantas medicinales*. 2011; 16(4): 313–323.
[Reference Source](#)
9. Monteiro JA, Ferreira Júnior JM, Oliveira IR, *et al.*: **Bioactivity and Toxicity of *Senna cana* and *Senna pendula* Extracts.** *Biochem Res Int*. 2018; 2018: 8074306.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Ravi B, Ramachandra Y, Sundara S, *et al.*: **Evaluación del potencial antihelmíntico de extractos de agallas de la hoja de *Terminelia chebula*. (Gaertn) Retz. (Combretaceae).** 2014; 6: 1–4.
11. Leite Ade S, Dantas AF, Oliveira GL, *et al.*: **Evaluation of toxic, cytotoxic,**

- mutagenic, and antimutagenic activities of natural and technical cashew nut shell liquids using the *Allium cepa* and *Artemia salina* bioassays. *Biomed Res Int*. 2015; **2015**: 626835.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
12. Borroto J, Trujillo R, De la Torre Y, *et al.*: **Actividad antimicrobiana y toxicidad frente a *Artemia salina* del extracto diclorometánico de raíces de *Morinda royoc* L.** *Revista cubana de plantas medicinales*. 2011; **16**(1): 34–42.
[Reference Source](#)
 13. Ávalos-Soto J, Treviño-Neávez JF, Verde-Star MJ, *et al.*: **Evaluación citotóxica de los extractos etanólicos de *Azadirachta indica* (A. Juss) sobre diferentes líneas celulares.** *Revista mexicana de ciencias farmacéuticas*. 2014; **45**(3): 39–44.
[Reference Source](#)
 14. Pino OP, Jorge FJ: **Ensayo de Artemia: Útil herramienta de trabajo para ecotoxicólogos y químicos de productos naturales** *Revista protección vegetal*. 2010; 34–43.
[Reference Source](#)
 15. Fernández-Calienes Valdés A, Mendiola Martínez J, Monzote Fidalgo L, *et al.*: **Evaluación de la toxicidad de extractos de plantas cubanas con posible acción antiparasitaria utilizando larvas de *Artemia salina* L.** *Revista Cubana de Medicina Tropical*. 2009; **61**(3): 254–8.
[Reference Source](#)
 16. Silva EMF, de Castro Nascimento RB, Barreto FS, *et al.*: **Estudo *in vitro* do potencial citotóxico da *Annona muricata* L.** *Revista de Ciências Farmacêutica Básica Aplicada*. 2015; **36**(2): 277–283.
[Reference Source](#)
 17. Miranda M, Cuellar A: **Mención en Productos Naturales y Terapéuticos. Maestría en Farmacia y Bioquímica. Escuela de Post-Grado.** Universidad Nacional de Trujillo. Cuba, 2003; 3–5.
 18. Seremet OC, Olaru OT, Gutu CM, *et al.*: **Toxicity of plant extracts containing pyrrolizidine alkaloids using alternative invertebrate models.** *Mol Med Rep*. 2018; **17**(6): 7757–7763.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 19. Meyer BN, Ferrigini NR, Putnan JE, *et al.*: **Brine shrimp: a convenient general bioassay for active plant constituents.** *Planta Med*. 1982; **45**(5): 31–34.
[PubMed Abstract](#) | [Publisher Full Text](#)
 20. Sandoval A: **Photos for the phytochemical analysis of the fruit leaves.** 2019.
<http://www.doi.org/10.6084/m9.figshare.8194580.v1>
 21. Sandoval A: **Toxicity percentage at different concentrations of hydro-alcoholic extract.** 2019.
<http://www.doi.org/10.6084/m9.figshare.7996598.v2>
 22. Ravi Shankara BE, Ramachandra YL, Rajan SS, *et al.*: **Evaluating the Anticancer Potential of Ethanolic Gall Extract of *Terminalia chebula* (Gaertn.) Retz. (Combretaceae).** *J Young Pharm*. 2014; **6**: 1–4.
[Publisher Full Text](#)
 23. Santos Pimenta LP, Pinto GB, Takahashi JA, *et al.*: **Biological screening of Annonaceous Brazilian Medicinal Plants using *Artemia salina* (brine shrimp test).** *Phytomedicine* 2003; **10**(2–3): 209–212.
[PubMed Abstract](#) | [Publisher Full Text](#)
 24. Cuadra P, Furrián M, Oyarzún A, *et al.*: **Biological activity of some Patagonian plants.** *Fitoterapia*. 2005; **76**(7–8): 718–721.
[PubMed Abstract](#) | [Publisher Full Text](#)
 25. Gouveia SC, Castilho PC: **Analysis of phenolic compounds from different morphological parts of *Helichrysum devium* by liquid chromatography with on-line UV and electrospray ionization mass spectrometric detection.** *Rapid Commun Mass Spectrom*. 2009; **23**(24): 3939–3953.
[PubMed Abstract](#) | [Publisher Full Text](#)
 26. Gouveia SC, Castilho PC: **Characterization of phenolic compounds in *Helichrysum melaleucum* by high-performance liquid chromatography with on-line ultraviolet and mass spectrometry detection.** *Rapid Commun Mass Spectrom*. 2010; **24**(13): 1851–1868.
[PubMed Abstract](#) | [Publisher Full Text](#)
 27. Leite A, Lima E, De Souza E, *et al.*: **Preliminary study of the molluscicidal and larvicidal properties of some essential oils and phytochemicals from medicinal plants.** *Rev Bras Farmacogn*. 2009; **19**(4): 842–846.
[Publisher Full Text](#)
 28. dos Santos Júnior HM, Oliveira DF, de Carvalho DA, *et al.*: **Evaluation of native and exotic Brazilian plants for anticancer activity.** *J Nat Med*. 2010; **64**(2): 231–238.
[PubMed Abstract](#) | [Publisher Full Text](#)
 29. Arcanjo DD, Albuquerque AC, Melo-Neto B, *et al.*: **Bioactivity evaluation against *Artemia salina* Leach of medicinal plants used in Brazilian Northeastern folk medicine.** *Braz J Biol*. 2012; **72**(3): 505–509.
[PubMed Abstract](#) | [Publisher Full Text](#)
 30. Guerra R: **Ecotoxicological and chemical evaluation of phenolic compounds in industrial effluents.** *Chemosphere*. 2001; **44**(8): 1737–1747.
[PubMed Abstract](#) | [Publisher Full Text](#)
 31. Cybulski WJ, Peterjohn WT, Sullivan JH: **The influence of elevated ultraviolet-B radiation (UV-B) on tissue quality and decomposition of loblolly pine (*Pinus taeda* L.) needles.** *Environ Exp Bot*. 2000; **44**(3): 231–241.
[PubMed Abstract](#) | [Publisher Full Text](#)
 32. Shen H, Tang Y, Muraoka H, *et al.*: **Characteristics of leaf photosynthesis and simulated individual carbon budget in *Primula nutans* under contrasting light and temperature conditions.** *J Plant Res*. 2008; **121**(2): 191–200.
[PubMed Abstract](#) | [Publisher Full Text](#)
 33. Simões R, Almeida S: **Phytochemical study *Bauhinia forficata* (Fabaceae).** *Biota Amazônia*. 2015; **5**(1): 27–31.
[Publisher Full Text](#)

Peer review discontinued Peer review at F1000Research is author-driven. Currently no reviewers are being invited. What does this mean?

Version 1

Reviewer Report 14 September 2020

<https://doi.org/10.5256/f1000research.20822.r69691>

© 2020 Gutierrez-Rebolledo G. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Gabriel Alfonso Gutierrez-Rebolledo

Academy of Toxicology, Department of Pharmacy, National School of Biological Sciences, National Polytechnic Institute, Mexico City, Mexico

Is the work clearly and accurately presented and does it cite the current literature?

- The objective of the work is innovative and is within the publishable objectives of the journal. However, although the techniques used were the correct ones to solve or fulfill the objective of the work, there are certain details that need to be refined in how much the specificities and calculations for the toxicological evaluation. The results were widely supported with updated scientific literature and related to the objectives of the experimental work.

Is the study design appropriate and does the work have academic merit?

- The proposed experimental design was adequate for the resolution of the objective and the initial hypothesis of the work. However, the arrangement within the text and its presentation format must be improved.

Are sufficient details of methods and analysis provided to allow replication by others?

- There were errors when making the calculations to obtain or prepare the stock solutions that would be used to obtain the concentrations to be tested for each extract. A thorough review of the calculations is recommended.

If applicable, is the statistical analysis and its interpretation appropriate?

- Although the correct or adequate statistical test was chosen for the type of variables and the proposed experimental design, these results of the statistical analysis were not properly represented in the graphs and mentioned in the text.

Are all the source data underlying the results available to ensure full reproducibility?

- The work can be reproducible without any doubt; however, the description of the methods, the way to calculate the results and to present / describe them should be improved.

Are the conclusions drawn adequately supported by the results?

- Yes, although they must be rewritten since they were not shown in a precise and concrete way, nor do they mention whether the main objective and the initial hypothesis of the work are accepted or denied with the results obtained.

Abstract:

In the abstract's background sub-section, please write a short line that mentions the importance of medicinal plant's pre-clinical toxicological studies. This line could be placed before mentioning the work's main objective. In this same sub-section please before describing the diverse Amazonian flora; please mention also that all these species have scientific supported biological activities, but non toxicological evaluation.

Please in the methods abstract's sub-section, correct the line in which it mentions that phytochemical analysis was carried out in the leaves, instead describe that it was made in every hydro-alcoholic leaf extract.

Please in this same abstract's sub-section avoid the extensive description of each method, this could be done in its respective section in the main text. This will leave you a lot more space to describe your findings in the abstract (the most out-standing ones).

Please rearrange the methods sequence as follows: biological material, hydro-alcoholic leaf extract preparation, and finally toxicity test.

In the results abstract's sub-section please re-write the first idea and you could better mentions that these two species (*Mauritia flexuosa* and *Musa* sp.) showed a concentration-dependent effect, since the way it is described, it is understood when reading it, that the higher concentration generated less toxicity when in the graphs it is observed for both species that the concentration of 1000 microliters / milliliter generated a toxicity value above 60% (highest).

Also in this same paragraph please mention for which of the three tested concentrations the other species *Cocos nucifera*, *Theobroma cacao* L. and *Coffea* sp. exerted a low toxicity over *Artemia salina*. Please correct the measurement units in which are mentioned the tested concentrations in the results abstract's sub-section change from $\mu\text{L}/\text{ml}$ to $\mu\text{g}/\text{ml}$.

Finally, in the conclusion abstract's sub-section, please re-write the whole paragraph, because as is mentioned in the authors and reviewers guidelines of this journal, conclusions shouldn't be suppositions, instead conclusions must mention if the work's main objective and its hypotheses were supported by the experimental findings, as well in this case you should mention of all the tested and studies vegetal species which were the less toxic, but also it should never be ensured that a substance is not toxic by having performed only one test of so many that are required in toxicological studies.

Introduction:

Please before start describing the bio-diversity of Amazonian jungle and rain forest, you should add a short paragraph in which you could describe some actual examples of used medical plants that resulted toxic through scientific techniques. Fuse this new paragraph with this idea "Studies have shown that the leaf extracts of some species are toxic for the human consumption because of the combination of their chemical compounds [8-11]".

After describing the phytochemical profile of the studied species, please add information related to how they are used by the population (ethnomedicinal applications and uses), and scientific supported biological activities (the most important at least).

In the last paragraph and before start describing *Artemia salina* experimental model importance, please add information in why is important to achieve pre-clinical toxicological studies in natural product's evaluations, mostly in medicinal plants.

Is it correct to ensure that this study in *Artemia salina* is sufficient to extrapolate its results to a genetically as different and remote species as the human being?

If the study is carried out in *Artemia salina*, it is ensured that the same study should no longer be carried out or similar in other animal species?

Materials and methods:

The method's sub-section entitled: "Source and husbandry of *Artemia salina*", should be rearranged after "Phytochemical analysis of plant samples" and before "Toxicity tests" sub-sections.

In the method's sub-section entitled: "Sources of plant samples", each of the registration numbers of the herbarium specimens (vouchers), and the person in charge who carried out the taxonomic identification, should be mentioned in the writing.

If the type of extract chosen was hydro-alcoholic, it should be mentioned in the sub-section entitled: "Preparation of plant samples", why this type of extract was chosen, as well as why it was chosen to work only with the leaves of the specimens, and also what is the water:ethanol ratio used, since the manuscript only mentions that the samples were placed by maceration in 96% alcohol.

In and after the sub-title "Preparation of plant samples" please you could change plant samples for hydro-alcoholic leaf extract. Do this correction in the rest of the manuscript from here forward.

If kind of studied extract was hydro-alcoholic, and then it was dried in a vertical rotary evaporator, please specify and add the temperature conditions used, since the extract has water in it, the conditions must be very specific to be able to evaporate that aqueous fraction, or it is done using a coupled vacuum pump to the rotary evaporator or with a subsequent lyophilization process, to avoid high temperatures and denature process of the metabolites present in the extract.

In the "Phytochemical analysis of plant samples" sub-section, this line is confusing "Briefly, each sample was subjected to solvents of increasing polarity, in order to obtain secondary metabolites according to their solubility...", does this mean that a chemical fractionation was carried out in a chromatography column using solvents of different polarities to group the secondary metabolites present in the extract? or that different solvents of increasing polarity were used to solubilize and restore the extract before performing the qualitative phytochemical tests? please explain better this matter.

Table 1 must be located and described in the Results section, mostly as phytochemical profile results. Please change the table 1 position.

Please, rearrange the "Ethical statement" position, this sub-section must be after "Phytochemical analysis of plant samples", and before the new location of "Source and husbandry of *Artemia salina*" sub-section, and after this last one you could put "Toxicity tests" sub-section, followed by "Statistical analysis" sub-section closing Materials and Methods manuscript's section.

Please, in the method's sub-section entitled: "Toxicity test", explain please how you did the calculations to obtain the test concentrations since no matter how much I repeat mine with the data that provide in the manuscript, the final concentration it is not the same value:

5µg of dried hydro-alcoholic extract + diluted in 5 ml of seawater equivalent to 10µg/ml
 $5\mu\text{g} / 5\text{ml} = 1\mu\text{g} / \text{ml}$

Or this one calculation could be the correct one: $50\mu\text{g} / 5\text{ml} = 10\mu\text{g} / \text{ml}$

50 µg of dried hydro-alcoholic extract + diluted in 5 ml of seawater equivalent to 100µg/ml
 $50\mu\text{g} / 5\text{ml} = 10\mu\text{g} / \text{ml}$

Or this one calculation could be the correct one: $500\mu\text{g} / 5\text{ml} = 100\mu\text{g} / \text{ml}$

500µg of dried hydro-alcoholic extract + diluted in 5 ml of seawater equivalent to 1000µg/ml

500µg / 5ml = 100µg/ml

Or this one calculation could be the correct one: 5000µg / 5ml = 1000µg/ml

This is an important point to correct or reassess, this does not mean that all the results obtained no longer serve, but rather readjust both their description, their discussion, conclusion and reclassify the extracts within the categories proposed in the description of the technique with the true values of the concentrations tested, which apparently were lower than those described in this version, which would be: 1, 10 and 100 µg/ml.

Results:

The first results that should be described in this section of the manuscript would be those related to the dry final weight of each of the hydro-alcoholic extracts, as well as the final yields of each one. Followed by the table of results of the phytochemical analysis. Place the table after the first paragraph of the results and not in the method section.

In the same description of the phytochemical profile results for each specie; also please mention which one extract has more chemical complexity, due to the presence of a greater amount of metabolites and also mention of what polarity nature they are mostly.

When describing your results, please mention what percentage of toxicity is related to each concentration evaluated, since it is confusing to read this section. A clear example of this reading and writing confusion is the next statement: "it can be observed that there is high toxicity (10 µg/ml), moderate (100 µg/ml) and low (1000 µg/ml) in *Mauritia flexuosa* and *Musa* sp; however, only low toxicity (1000 µg/ml) is evidenced in *Cocos nucifera*, *Coffea* sp. and *Theobroma cacao* L (see Underlying data)", but how can this possible if when I see the graphic what is understood is the following: for *Mauritia flexuosa* toxicity percentages for each tested concentration approximately were 50% for 10µg/ml, 60% for 100µg/ml and 70% for 1000µg/ml, same behavior observed in the graphic for *Musa* sp., so why do you considerer 1000µg/ml as a low toxicity?

In the graphic all abbreviations must be explained in the footnote, so please describe what means TA, TB, and TM.

In the result's discussion please in addition to classifying each extract according to its toxicity, relate this observed effect to what type of metabolites are present in each, and support this idea by seeking information of toxicological evaluations carried out for each family of metabolites in the same plant species, or within plants of the same scientific genre or taxonomical family.

Both in the graph and described in the text when mentioning the results obtained, the significant or statistical differences between the experimental groups must be mentioned and exemplified in the graph using superscripts.

Conclusions:

The last paragraph of this manuscript should be completely rewritten because of a conclusion in an experimental work:

1. must be concrete and related to the objective / hypothesis of the work.
2. should not be referenced, so this current paragraph of the manuscript would not go into conclusions but rather in discussions of the results.

This entire paragraph: "Health treatments with medicinal plants are cheap and accessible to the

population; however, their indiscriminate use is a risk due to the toxicity of some compounds within the plant. For this reason, it may be useful to study plant extracts with the aim of demonstrating the therapeutic or toxic action of their active components, using *Artemia salina* as an evaluation method. In this context, the toxicity of hydroalcoholic extracts of fruit leaves from the Peruvian Amazon was evaluated. It can be concluded that the obtained results are in accordance with other studies that examined different extracts as reported by Simões and De Almeida³³, indicating that if a sample is nontoxic to *Artemia salina*, then its effects will also be similar to humans.”, must be in the result’s discussions sub-section in the Result’s section.

As conclusions of their work, they could mention which were the main types of secondary metabolites that were found in the various extracts of the hydro-alcoholic type, as well as mention how these are related to the toxicity shown by each species, and finally, mention within the classification system used which species were most toxic.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Toxicological and pharmacological pre-clinical evaluation of natural and synthetic compounds

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research